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THE PREPARATION AND ABSORPTION SPECTRA OF FIVE PURE CAROTENOID PIGMENTS[1, 2]

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(WITH FOUR FIGURES)

Introduction

For several years one of the objectives of this laboratory has been the development of a satisfactory spectroscopic method of analysis of simple extracts of biological materials for each of the principal carotenoid pigments. Efforts are being made to devise methods by which several pigments in a single extract may be analyzed with as little manipulation as possible. The degree of simplicity will depend upon the number and nature of the pigments present and the ease of extraction, as well as other factors which may vary according to the material.

The work reported here includes the isolation and purification of five of the more commonly occurring carotenoids, the accurate determination of their absorption spectra, and the effect of variation of the spectral region isolated on the numerical values of the absorption coefficients. These spectra are expected to provide the fundamental constants of a system of quantitative spectroscopic analysis which will include all of these pigments. The pigments studied were alpha-carotene, beta-carotene, cryptoxanthol, luteol, and zeaxanthol. Three carotenol esters were also isolated. Similar studies on other pigments are expected to follow.

The isolation and purification of these two carotenes has been discussed in considerable detail (**12, 15, 17**). The most authoritative and detailed discussions of the carotenols are those of Strain (**16**) and Zechmeister (**19**). Comparison shows that in most cases agreement with their results is good; however, a few improvements in preparative technique are reported here.

[1] Studies on the Carotenoids 1: The first of a group of papers on their spectrometry and chemistry.

[2] Joint contribution from the Purdue Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

The usual method for isolation of these pigments has been chromatography of the unsaponifiable fraction of plant extracts followed by repeated crystallization. Recrystallization of the carotenoids is usually accomplished by the addition of a liquid in which the pigment is only slightly soluble to a concentrated solution of the pigment.

Methods

The instability of the carotenoids toward heat, oxygen, and light was recognized. While in solution, the pigments were kept at refrigerator temperature in the dark and under an atmosphere of nitrogen as much as possible. Crystalline preparations were dried at room temperature *in vacuo* and stored during study in an easily accessible vacuum chamber kept in darkness at 0° C. at a pressure of about 15 μ of mercury (measured by a "Tru-Vac" gauge). Pigments were stored for longer periods in evacuated glass tubes in a refrigerator.

Manipulation of solutions was as rapid as possible and in a nitrogen atmosphere whenever feasible. Solutions were never heated above 40° C. Reduction in volume was accomplished by evaporation under reduced pressure. The possibility of alteration of the pigments by residues from cleaning of glassware by acid dichromate solution was eliminated by the use of a trisodium phosphate rinsing solution.

The magnesium oxide (Micron Brand no. 2641, California Chemical Co.) suggested by STRAIN (**15**) was used, diluted by one part of "Hyflo Super Cel" (Johns-Manville). Before use, the columns were flushed by a current of nitrogen. Water jackets were used to keep the column temperature below 10° C.

PURIFICATION OF SOLVENTS

All solvents used were purified as described below, distilled in all-Pyrex apparatus with non-lubricated joints, and stored in darkness.

Petroleum ether. A commercial grade of solvent was exhaustively treated with alkaline potassium permanganate solution and redistilled.

Hexane. The hexane used in this work was obtained from a commercial grade of petroleum ether with a labelled boiling range of 65° to 67° C. This was treated exhaustively with alkaline permanganate and redistilled. Only the portion which distilled between 65° and 67° C. was used.

Ethanol. Commercial 99.5 per cent. ethyl alcohol was refluxed with zinc dust and solid sodium hydroxide for one hour, then distilled.

Carbon disulphide. The "Analytical Reagent" grade was redistilled shortly before use.

Methanol. Commercial synthetic methanol was treated in the same manner as was the ethanol.

Ether. Commercial ether was redistilled after standing for a week over solid sodium hydroxide.

Chloroform. The "Analytical Reagent" grade was redistilled shortly before use.

Purification of pigments

ALPHA-CAROTENE.—The alpha-carotene was obtained from commercial samples of carrot root "crystalline carotene" (Nutritional Research Assoc., Inc.), which consisted of a mixture of alpha- and beta-carotenes, together with various impurities. The preliminary purification was accomplished by the use of chromatographic adsorption on magnesium oxide. The pigment mixture was dissolved in a minimum amount of petroleum ether (40°–60° C.) and adsorbed on the column. After development by thorough washing with petroleum ether, the zones containing alpha- and beta-carotenes were rather clearly defined and separated from the impurities. The column was removed by sections, and the alpha-carotene eluted from the adsorbent with 5 per cent. ethanol in petroleum ether. The ethanol was washed from the petroleum ether and the pigment adsorbed on a second column. This adsorption process was repeated four times, until there was no visible impurity on the column.

The alpha-carotene solution in petroleum ether (34°–40° C.) from the last column was passed through a fine sintered-glass filter to remove dust and other insoluble contaminants and evaporated to dryness. The pigment was taken up in a minimum amount of carbon disulphide, and crystallized by the addition of two volumes of ethanol. The crystals were removed by filtration through a Berlin crucible in an atmosphere of nitrogen. After being washed with a small volume of cold ethanol, the crystals were transferred to a glass capsule and dried for 24 hours. Four crystallizations were necessary to obtain a product having absorption coefficients which remained constant after an additional crystallization.

BETA-CAROTENE.—The beta-carotene was purified from the same source and with the same technique as described above for alpha-carotene. After the fourth crystallization, no increase in the specific absorption coefficients occurred.

ZEAXANTHOL.—This pigment is the principal carotenol of corn grain (**19**). It also occurs in *Capsicum annuum* (**19**), *Physalis alkekengi* (**19**) and in small quantities in leaves (**16**).

Yellow corn grain and two varieties of *Capsicum annuum,* ripe pimento peppers and commercial powdered paprika, were investigated as possible sources by methods similar to those given below. Only traces of zeaxanthol were obtained in these cases; therefore the red calyx of the Physalis plant was examined.

It has been observed in this laboratory that the use of dichloroethane as

a solvent in chromatographic adsorption, as suggested by STRAIN (**16**), resulted in columns that filtered and dried slowly. The zones tended to run together if attempts were made to separate them before the column was dry. This solvent is somewhat difficult to remove *in vacuo* at room temperature. Adsorption of the pigments from it was such that long columns were necessary and 12 to 30 hours were at times needed to complete development of a column. It was observed that diethyl ether was a very good solvent for chromatography of carotenols on magnesium oxide. It has nearly as high solvent powers for the pigments as dichloroethane, is easily purified to the necessary extent, and is readily removed by evaporation *in vacuo*. When ether is used as the solvent, the zones may be separated almost immediately after the level of the ether used for washing reaches the top of the column. The carotenes are not adsorbed from ether; most carotenols are adsorbed from ether to nearly the same extent as are the carotenes from petroleum ether; cryptoxanthol is adsorbed weakly. Ether was used in all subsequent adsorption columns for carotenols. Ethanol or ethanol-ether solutions were used to elute the carotenols. Traces of alcohol in the ether used for chromatography must be avoided.

Physalis calyx (217 gm.) was used in the first experiment. The dry ground material was extracted with low-boiling (34°–40° C.) petroleum ether. The solvent was evaporated under reduced pressure and the residual deep red oil was saponified 3 days at room temperature. A preliminary adsorption yielded 5 zones. The two which were most likely to contain zeaxanthol were combined and readsorbed. A zone was separated which, after another adsorption, yielded 68.6 mg. of crude zeaxanthol, m. p. 194–196° C. Three recrystallizations from chloroform and methanol gave a product that melted at 206.6° C. All melting points were determined on a microscope hot stage described by ZSCHEILE and WHITE (**22**).

A second lot of Physalis (215 gm.) was ground, extracted, and saponified as above. Behavior of the pigments on subsequent adsorption from ether indicated that the saponification was incomplete. After the adsorption column had been thoroughly washed with ether, 3 zones remained adsorbed, the lowest of which continued into the filtrate.

Zone 1 (at the top) was eluted, transferred to sufficient chloroform to make a saturated solution, and $2\frac{1}{2}$ volumes of methanol were added slowly. Zeaxanthol (28 mg.) crystallized in glittering plates that melted at 201.2° C. Two recrystallizations from chloroform-methanol raised the melting point to 206° C.

The pigment from zone 2 was crystallized as above. A yield of 95 mg. was obtained (m. p. 143° C.). Recrystallization did not raise the melting point. The absorption spectrum was of the beta-carotene type. The specific absorption coefficient at 4525 Å in hexane was 171. The specific absorption

coefficient calculated for zeaxanthol monopalmitate is 174. KARRER and SCHLIENTZ (**5**) reported a preparation of zeaxanthol monopalmitate to melt at 148° C. A sample (6.8 mg.) of the pigment was hydrolyzed and about 1.5 mg. of zeaxanthol was obtained (m. p. 206.4° C.). These results indicate a monoester of zeaxanthol.

The pigment from zone 3 was transferred to petroleum ether and readsorbed. The pigment from the uppermost zone of this column was crystallized from chloroform-methanol. A product (704 mg.) that melted at 97.5° C. was obtained. This corresponds to physalien, which ZECHMEISTER reported to melt at 98.5° to 99.5° C. (**19**) and to be zeaxanthol dipalmitate.

Another sample of physalien (twice recrystallized, m. p. 100.6° C., 121 mg.) was saponified in ether at room temperature. After washing with water, the solution was evaporated to dryness under reduced pressure. The residue (48.6 mg.) melted at 206.5° C. The recrystallized product weighed 38 mg., melted at 208.0° C., and its specific absorption coefficient at 4525 Å in ethanol was 232. The substance was dried at 84° C. at a pressure of 15 μ of mercury for 3 hours. This raised the absorption coefficient to 249 and indicated that 6.6 per cent. of the original weight was solvent.

CRYPTOXANTHOL.—This compound is the principal vitamin A precursor in corn grain (**7**).

The cryptoxanthol was isolated from miscellaneous residues from the isolation of zeaxanthol from Physalis described above. These fractions were combined and adsorbed from ether on magnesium oxide. Six zones, including the filtrate, were found. The uppermost was discarded since it probably contained impurities and oxidation products. Zeaxanthol was isolated from the next zone. The third zone was small and was not examined. The fourth zone yielded small amounts of luteol. The fifth zone was not contiguous with the fourth and yielded 10 mg. of cryptoxanthol, which melted at 167.3° C. Recrystallization from chloroform by addition of three volumes of ethanol raised the value to 169° C. The pigment had an absorption spectrum of the beta-carotene type. The sixth zone (filtrate), light in color, was discarded.

During the separation of the zeaxanthol esters previously described, a zone separated below the physalien zone. Attempts to crystallize the pigment at 5°–10° C. produced deep red crystals, which melted to a red oil at room temperature. Saponification of this oil yielded a pigment melting at 167° C. which was identified as cryptoxanthol. The low-melting solid, therefore, may have been a cryptoxanthol ester, in consideration of its position on the adsorption column and its product after saponification. All of the cryptoxanthol preparations obtained were combined and recrystallized three times. Weight 8.6 mg., m. p. 165.6° C.

LUTEOL.—Luteol is the principal carotenol of leaves (**15**). Corn seed-

lings were used as a source for the isolation. The ground tissue, dried below 45° C., was extracted successively with petroleum ether, ether, methanol, and acetone. The pigments were transferred to ether and saponified at room temperature for 48 hours. The ether solution was washed with water and concentrated. Waxy material was removed on a chilled filter.

The carotenol pigments in the ether solution were chromatographically separated from the carotenes. The adsorption process was repeated until

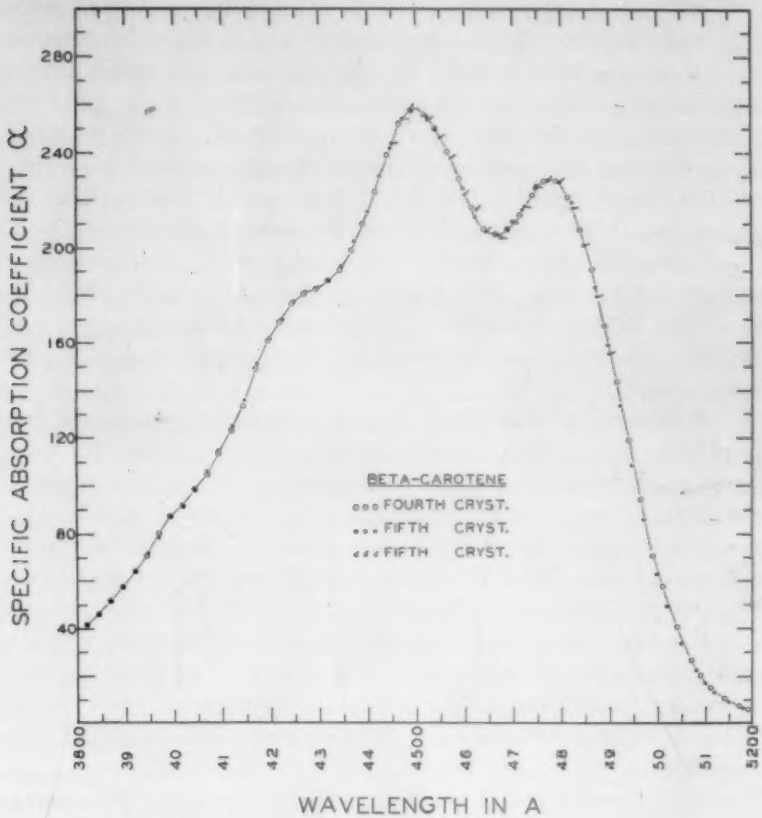

FIG. 1. Absorption spectrum of beta-carotene in hexane solution.

only the luteol band appeared. The eluate was then washed free from ethanol and the remaining ether solution evaporated to dryness. The pigment was taken up in a minimum amount of warm carbon disulphide. To this solution was added about 1 ml. of ethanol for every 10 mg. of luteol present. The solution was warmed and nitrogen was blown onto the surface of the solution to evaporate carbon disulphide until crystals just started to form. At this point the solution was warmed slightly to dissolve all of the

luteol. The solution then remained at 0° C. under nitrogen. After standing 24 hours the crystals were filtered and the product recrystallized three times (m. p. 150.8° C.). Luteol crystallized very easily from chloroform and ethanol but was found to be unstable in this solution, as noted by STRAIN (**16**).

When luteol was crystallized from carbon disulphide-ethanol solution, approximately 10 per cent. of solvent was retained. This is in agreement with STRAIN's work (**16**). It is necessary to heat luteol at 84° C. for 4 hours under a high vacuum to remove this solvent. Almost identical absorption curves were obtained for a luteol sample heated 4 hours and for one heated 8 hours.

A sample from alfalfa leaves was obtained by chromatography and recrystallization of a commercial "xanthophyll" preparation (American Chlorophyll Co.).

SPECTROSCOPIC METHODS

The absorption spectra of the pigments were determined by means of a new photoelectric spectrophotometer recently employed for similar measurements on the chlorophyll components (**21**). In principle this instrument is similar to that described by HOGNESS, ZSCHEILE, and SIDWELL (**3**). The improved instrument will be described in detail at a later time. It employs a large Müller-Hilger Universal Double Monochromator with crystal quartz optics. A Mazda incandescent filament lamp was the source of radiation. ZSCHEILE and COMAR (**21**) reported the precision error of measurement of $\log_{10}\frac{I_o}{I}$ values by this instrument to be $\pm$ 0.5 per cent. for $\log_{10}\frac{I_o}{I}$ values of 0.200–0.800, with total precision errors, including weight and volumetric technique, not in excess of $\pm$ 0.6 per cent.

For these measurements, all three slits were of uniform width with the exit slit 6 mm. in height. For all pigments except zeaxanthol, the slits were 0.08 to 0.06 mm. in width; they isolated spectral regions of 8.4 to 18 Å over the region studied (3800–5200 Å). The widths of the spectral regions isolated were calculated according to the method of HOGNESS, ZSCHEILE, and SIDWELL (**3**). In the region of maximum absorption, the regions isolated were approximately 12 to 16 Å. The two curves for zeaxanthol were obtained with slits from 0.05 to 0.15 mm. The regions isolated were thus 5 to 44 Å. In the region of maximum absorption, the regions isolated were approximately 10 to 30 Å. Measurements were made at intervals of 20 or 25 Å.

The absorption spectra reported here are expressed in terms of the specific absorption coefficient, α:

$$\alpha = \frac{\log_{10}\frac{I_o}{I}}{cl}$$

I_o = intensity of radiant energy transmitted by solvent-filled cell.
I = intensity of radiant energy transmitted by solution-filled cell.
c = concentration in grams per liter.
l = thickness of solution layer in centimeters.

The concentration is expressed in grams per liter because it is expected that these spectra will be used for quantitative analysis of the pigments. The cell lengths and concentrations were adjusted to keep the $\log_{10} \frac{I_o}{I}$ values between 0.200 and 0.800.

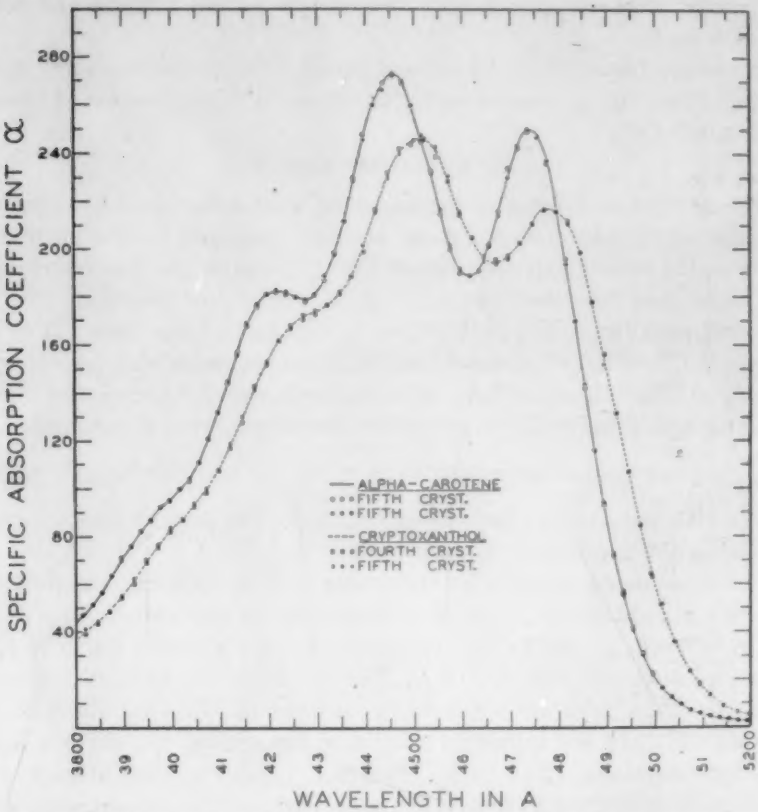

FIG. 2. Absorption spectra of alpha-carotene and cryptoxanthol in hexane solution.

ABSORPTION SPECTRA

Figure 1 presents absorption data for beta-carotene in hexane solution. To illustrate the overall precision of the entire experimental procedure, three entirely separate sets of spectrophotometric observations are presented for three weighed samples from two different crystallizations. Observations as well as weighings were made by two different observers.

In figure 2 are the curves for alpha-carotene and cryptoxanthol in hexane solution. Two sets of data are included for each pigment. Two different operators obtained the data for alpha-carotene.

Similar data for luteol and zeaxanthol in ethanol solution are presented in figure 3. Each set of data for zeaxanthol represents a different crystal-

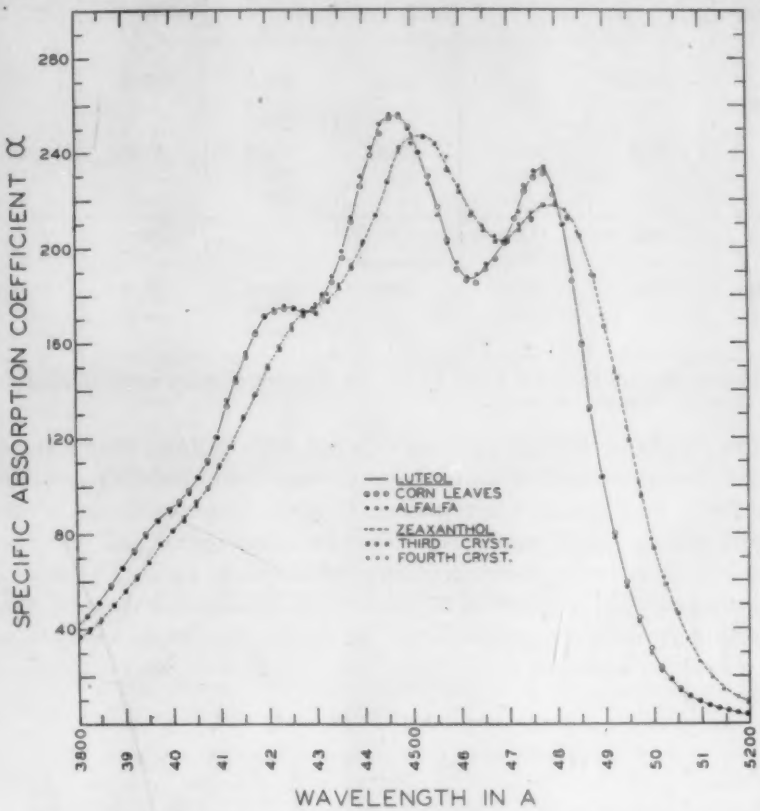

FIG. 3. Absorption spectra of luteol and zeaxanthol in ethanol solution.

lization. In the case of luteol, the samples for each set of data were from a different source of material, corn and alfalfa leaves. These curves are in excellent agreement with the absorption spectrum of luteol isolated from barley leaves as reported by STRAIN (**16**).

Tables I and II present the numerical values of absorption coefficients at maxima and minima.

EFFECT OF SLIT WIDTH AND TESTS FOR SCATTERED RADIATION

A further study was made of the effect of slit width on the absorption values obtained for beta-carotene so that direct comparison could be made

TABLE I

ABSORPTION VALUES OF CAROTENOIDS IN HEXANE SOLUTION

	ALPHA-CAROTENE		BETA-CAROTENE		CRYPTOXANTHOL	
	λ (Å)	a	λ (Å)	a	λ (Å)	a
Maxima	4220	182.0*	.....	.....	.....	.....
	.....	183.0	.....	.....	.....	.....
	4460	273.0	4500	257.5	4515	246.0
	.....	272.0	.....	257.5	.....	246.5
	.....	.....	.....	258.2	.....	.....
	4740	249.0	4780	228.2	4780	216.0
	.....	249.0	.....	228.2	.....	217.0
	.....	.....	.....	228.2	.....	.....
Minima	4280	178.0	.....	.....	.....	.....
	.....	179.0	.....	.....	.....	.....
	4615	189.0	4665	206.0	4670	194.0
	.....	189.0	.....	205.8	.....	196.2
	.....	.....	.....	206.0	.....	.....

* Values reported in tables I and II are for different samples corresponding to the curves shown in figures 1, 2, and 3.

with the results of MILLER (11) on this subject. MILLER found that the values of absorption coefficients for beta-carotene were extremely sensitive to slit widths. In figure 4, a comparison is made between MILLER's results obtained with a single monochromator with glass optics and our results obtained with a double monochromator with quartz optics. Our experiments covered a much wider set of slit conditions than did those of MILLER and strikingly different results were obtained. Our measurements were made when slits varied in width from 0.015 to 0.90 mm. corresponding to a

TABLE II

ABSORPTION VALUES OF CAROTENOIDS IN ETHANOL SOLUTION

	LUTEOL		ZEAXANTHOL	
	λ (Å)	a	λ (Å)	a
Maxima	4230–4250	175.0	.....	.....
	.....	175.8	.....	.....
	4465	255.0	4520	247.0
	.....	256.0	.....	248.0
	4775	231.0	4790	220.0
	.....	234.5	.....	218.0
Minima	4280	173.7	.....	.....
	.....	174.2	.....	.....
	4625	185.7	4690	204.0
	.....	188.0	.....	203.0

spectral region isolated of 3 to 225 Å. Results were those which might be expected from the nature of the absorption curve and from the assumption that no scattered radiation was present from other regions of the spectrum. Absorption values decreased at the maxima and increased at the minima when the spectral region isolated became sufficiently great. These changes were relatively small and dependent upon the average absorption over the

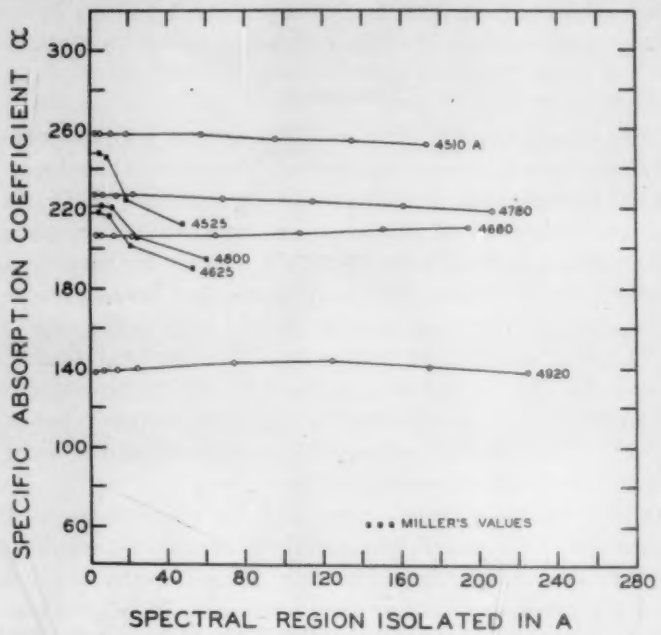

FIG. 4. Effect of width of spectral region isolated on the specific absorption coefficient of beta-carotene at selected wavelengths.

region considered. It is evident from figure 4 that constant absorption values were obtained under the following conditions:

4500 Å (maximum)	slits	0.015–.30 mm.	spectral region	3–58 Å
4665 Å (minimum)	"	0.015–.50 mm.	" "	3–108 Å
4780 Å (maximum)	"	0.015–.30 mm.	" "	3–69 Å
4920 Å (slope)	"	0.015–.06 mm.	" "	3–14 Å

The data of MILLER are calculated directly from his data for beta-carotene [fig. 5 (**11**)] and from the region isolated as given by him for his monochromator [table III (**11**)].

Since the 4358 Å line from the mercury spectrum is the only well-isolated line in this region which would permit an extensive study of slit widths without inclusion of other lines, it was the only one studied. Absorption coefficients of beta-carotene solutions at 4358Å remained constant over a

slit width range of 0.06–0.50 mm. when the incandescent lamp was replaced by a mercury arc source.

Absorption measurements were made with an incandescent source at 4500, 4665, and 4780 Å when the following glass filters were placed in front of the entrance slit of the monochromator: Jena BG7 (absorbs from 6400 Å to the infra-red limit of the photocell), Corning 385 (absorbs below 3700 Å), Corning 585 (absorbs from 5000 to 6900 Å), and all 3 filters in series. No variation was observed from the values obtained without a filter.

Discussion

The absorption spectra of carotenoids in several solvents were studied before the final choice of solvent was made. The ether-ethanol mixture that has been used considerably as a solvent for carotenoids (**11, 14**) was found to produce less resolution of the principal bands of the carotenes (lower ratios of the maxima to the minima) than was the case for hexane. Moreover, absorption curves for hexane solutions of alpha- and beta-carotenes diverge appreciably more on the slopes toward the red than is the case for ether-ethanol. This is of considerable importance from the analytical viewpoint. Ether-ethanol has the further disadvantage of being a mixed solvent and this factor would make it very difficult to employ in certain types of analytical procedure. The curve of zeaxanthol in ether-ethanol is very close to that reported here for the ethanol solution.

Several hydrocarbon solvents were used for observations of the beta-carotene spectrum. Compared with results in hexane, the maxima for heptane solutions are shifted approximately 25 Å toward the red. With petroleum ether fractions distilling over a range of 20° C., we were unable to obtain reproducible spectroscopic results. This difficulty is attributed to lack of uniformity in the composition of the solvent. CARTER and GILLAM (**1**) have found that the position of the maxima of beta-carotene varies with the boiling range of the petroleum ether solvent. Very minor differences between results in hexane and pure 2,2,4-trimethylpentane ("iso-octane") were found for both alpha- and beta-carotenes. Hexane is an excellent solvent from all viewpoints. It is equal to "iso-octane" from the spectroscopic and analytical viewpoints and has the further advantage of being available at a comparatively low price. We consider it well adapted to routine analytical work.

The numerical values given by MILLER (**11**) for the width of spectral regions isolated at various wavelengths are approximately twice the results that would be calculated by his method from the dispersion data given by HOGNESS, ZSCHEILE, and SIDWELL and by the manufacturer (**3**) for the same type of monochromator. His values (plotted in figure 4) are 1½ times as great as would be obtained if calculated by the method employed here.

It is apparent from the results shown in figure 4 that the double monochromator is far superior, insofar as scattered radiation is concerned, to the single monochromator as employed by MILLER. The presence of scattered radiation from another region of the spectrum would explain his results obtained with different slit widths. The results with the 4358 Å mercury line and with previously filtered incandescent radiation are further evidence

TABLE III

SPECIFIC ABSORPTION COEFFICIENTS AT HIGHEST MAXIMUM

SOLVENT	ALPHA-CAROTENE	BETA-CAROTENE	CRYPTO-XANTHOL	LUTEOL	ZEA-XANTHOL
Hexane	270 (13)* 273 (6) 272	275 (13) 302 (6) 258	246		
20 per cent. ether in ethanol	263 (14) 258 (11) 271 (18)	249 (11) 251 (14) 255 (18)		258 (16, p. 82)	
Carbon disulphide	202 (14) 218 (6)	194 (14) 194 (9) 243 (6)	204 (16, p. 106)	216 (8) 191 (16, p. 79)	223 (8) 200 (13) 185 (16, p. 106)
Chloroform		220 (2)		180 (2) 238 (16, p. 80)	149 (2)
Petroleum ether (b.p. 55–65° C.)	279 (17)	253 (17)			
Ether		208 (4)		248 (4) 260 (16, p. 82)	
Ethanol			247 (16, p. 105)	221 (13) 254 (16, p. 84) 255	249 (16, p. 102) 236 (13) 248

* Most of these values were approximated from graphs in the papers quoted. The values without reference numbers are averages of those presented in this paper.

that scattered radiation is negligible under the conditions of measurement employed in this laboratory.

It is evident that all of the spectra discussed are of one of two types, those possessed by carotenoids of either the alpha-carotene or the beta-carotene type of molecular structure. It should be possible to analyze a mixture quantitatively for each of any two compounds having such different types of spectra. Wavelengths at which their absorption values are widely different may be employed for simultaneous equations, the solution of which

will give both the total concentration and that of each component. Wavelengths at which the absorption curves cross or coincide may be employed for total concentration (**10, 20**). These applications will be developed in later papers. It will be observed that the spectrum of cryptoxanthol is quite similar to that of beta-carotene. The former is lower by a nearly constant factor. The percentage composition of pure mixtures of these two pigments cannot be determined by present spectrophotometric methods, since in no region do the absorption curves separate from each other by more than 5 per cent.

The values of the absorption coefficients at the highest maxima, as obtained by workers in this field, are assembled in table III. These values have been recalculated in terms of specific absorption coefficients to facilitate comparison. It will be seen that the values obtained in this investigation are in fair agreement with the majority of the others.

Summary

1. The following carotenoids were isolated and purified by chromatographic and crystallization methods accompanied by spectroscopic control: alpha-carotene, beta-carotene, cryptoxanthol, luteol, and zeaxanthol.

2. The absorption spectrum of each was determined from 3800 to 5200 Å by a photoelectric spectrophotometric method.

3. Absorption coefficients were found to be relatively insensitive to changes in slit width, except in regions where absorption values change rapidly with wavelength. Experiments with mercury arc radiation and filters also indicate that scattered radiation is negligible.

4. The absorption values given are considered suitable for a spectroscopic basis of analysis for individual carotenoids.

5. These values are compared to those obtained by other workers. Differences due to solvent are discussed.

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EFFECT OF SALINE SUBSTRATE ON HOURLY LEVELS OF CARBOHYDRATES AND INORGANIC CONSTITUENTS OF BARLEY PLANTS[1]

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(WITH SIX FIGURES)

Introduction

Salinity considerations are likely to be an important feature of irrigation agriculture wherever it is practiced. The fact that a depressed growth of many plants results from even moderate accumulations of chloride and sulphate salts in soil solutions has a highly practical significance. From the standpoint of the investigator, it is particularly desirable that the mechanism of salt injury be as clearly understood as our present-day insight into biological processes will permit.

The investigation reported in this paper had the two-fold objective of determining (a) if any relationship existed between salt accumulation in the barley plant and the hourly march or levels of carbohydrate accumulation; and (b) the relation between cyclic changes in transpiration rates and the accumulation of the chemical constituents of the substrate in the shoots of barley plants.

Measurements were made of the concentrations of sugars, starch, and inorganic constituents in 5-week-old barley plants at 4-hour intervals over a 24-hour cycle. The three sets of plants compared were grown in sand cultures supplied with solutions as follows: (a) base nutrient only; (b) base nutrient plus 100 milliequivalents per liter of added chloride; and (c) base nutrient plus 200 milliequivalents per liter of added sulphate.

Materials and methods

Barley was used as the experimental plant; seed of the Sacramento variety was supplied by the University of California. The plants were grown at Riverside, California, in the large, out-of-door, automatically-operated sand culture equipment described by EATON (7). The seeds were planted on April 2, 1940, at a depth of one inch, spaced one inch apart in the 30-inch rows, and thinned on April 23 to fifteen plants per row. The sixteen rows of barley per bed were spaced 10.5 inches apart with end rows set 5.5 inches from the ends of the beds.

The composition of the three culture solutions is shown in table I.

The nutrient solutions were automatically pumped onto the beds twice

[1] Contribution no. 20 of the U. S. Regional Salinity Laboratory, Bureau of Plant Industry, Riverside, California, in cooperation with the Western States.

TABLE I

COMPOSITION OF CULTURE SOLUTIONS*

SOLUTION	MILLIEQUIVALENTS PER LITER								PARTS PER MILLION			CONDUCTANCE $K \times 10^5$ AT 25° C.	OSMOTIC CONCENTRATION
	Ca	Mg	Na	K	Cl	SO_4	PO_4	NO_3	Cl	SO_4	TOTAL IONS		
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*p.p.m.*	*p.p.m.*	*p.p.m.*		*atm.*
Base nutrient	6.2	5.4	1.9	3.2	4.0	4.6	0.6	8.0	.....	.....	1,071	178	0.736
100 m.e./L. of Cl	26.2	35.4	41.9	3.2	104.0	4.6	0.6	8.0	3,550	.....	6,532	1,172	4.192
200 m.e./L. of SO_4	26.2	85.4	101.9	3.2	4.0	204.6	0.6	8.0	.....	9,600	14,361	1,388	4.158

* Iron was supplied by 0.2 per cent. of magnetite mixed with the sand. Trace elements were supplied to give: boron, 0.6 p.p.m.; Mn, 0.2 p.p.m.; and, from impurities in the salts, approximately 0.2 p.p.m. of zinc.

daily until May 6, after which time the flushing hours were 7:00 A.M., 9:00 A.M., and each hour thereafter until and including 6:00 P.M. Each culture solution was maintained at pH 6.0 by the addition of small amounts of concentrated nitric acid (**8**).

All cultures were supplied with base nutrient only from April 2 to April 5, 1940. On April 5 the salt beds received one-fifth of the amount of salt that was to be added to bring them to the above-stated concentrations. The second, third, fourth, and fifth increments of salt were added to the salt beds on the 11th, 12th, 14th, and 17th of April, respectively.

The plants, tops only, were harvested over a 24-hour period. Two rows of plants were cropped from each bed every four hours beginning at 4:00 A.M. on May 10th, and ending at 4:00 A.M. on May 11th, 1940. The second and ninth rows were removed first, the third and tenth at 8 A.M., continuing on in sequence until all but the first and sixteenth rows (outside rows) had been taken out. These latter were removed on the third morning and used as preliminary test material in the analyses.

Each of the rows was harvested, weighed, and analyzed separately. After a fine chopping of each row, a 10-gram (fresh weight) sample was put up in boiling alcohol for sugar analyses. The remainder of each row sample was dried to determine the percentage of moisture, and also to furnish the dry matter on which the inorganic analysis and starch were to be determined.

The designations "reducing sugars," "sucrose," and "starch" are used in the conventional and well-recognized manner without assuming that the entire amount of the reducing materials measured were these specific substances. Technique and reagents were carefully standardized to insure a high degree of dependability in comparisons between treatments and hours of harvest.

Sugars

Reducing sugars.—The finely-chopped, 10-gram samples of plant material were dropped into boiling ethyl alcohol of such strength that the final concentration would be approximately 80 per cent. and the boiling continued for approximately five minutes to insure complete penetration of the alcohol and the cessation of protoplasmic and enzymatic activity.

For extraction, 125 ml. of 80 per cent. ethyl alcohol was added to the sample and the whole placed in a Soxhlet extractor of the newer type in which the extracting alcohol in the thimble approaches the temperature of the liquid in the receiving flask.

The alcohol was evaporated from the extract on a steam bath, the resulting aqueous extract cleared with neutral lead acetate, and deleaded with disodium hydrogen phosphate according to Hassid's method (**12**). The mixture was then made to 100 ml. and filtered through a dry filter discard-

ing the first 2 or 3 ml. of filtrate. Five-ml. aliquots were used for the determination of reducing sugars following a modified HARDING and DOWNS method described by VAN DER PLANK (**18**).

TOTAL SUGARS.—The pH of a 50-ml. sample of the foregoing solution was adjusted to 4.9 with a few drops of 10 per cent. acetic acid, 2 to 4 drops of a 1 per cent. solution of WALLERSTEIN invertase scales added, and the solution then hydrolyzed overnight at room temperature (**12**). The following morning the solution was made to 100 ml. and filtered through a dry filter discarding the first 2 or 3 ml. of filtrate. Five-ml. aliquots were used as before for the determination of reducing substances.

SUCROSE.—This value was obtained by subtracting the reducing-sugar value from the total-sugar value.

STARCH

Fresh samples of the plant material were dried and ground, then redried in an oven at 110° C. for 2 hours, and allowed to cool in a desiccator before weighing. When possible, a 5-gram sample was used for analysis. The procedure of HASSID, *et al.* (**13**), was followed for the solubilization and extraction of starch. The extract was hydrolyzed overnight with pancreatin-free plant diastase at room temperature. After hydrolysis the solution was cleared according to the method described by HASSID (**12**), and reducing sugars again determined as before.

INORGANIC ANALYSES

The official methods of the Association of Official Agricultural Chemists (**1**) for plant materials were followed for the determination of calcium, magnesium, total sulphur, total phosphorus, and total nitrogen. Sodium was determined by the uranyl zinc acetate method, and potassium by a cobaltinitrite procedure.

Weather conditions

Overcast skies and relatively cool weather predominated during the period preceding the day of sample collection. Between April 16 and May 9 the average maximal temperature was 76.8° F. and the average minimal, 49.3° F. The average relative humidity at noon was 41 per cent. and average wind movement was 43.1 miles per 24 hours.

On the day of harvest, May 10, 1940, the sky was clear at 8:00 A.M., partially cloudy at noon and also at 4:00 P.M. On May 11, the sky was clear by 8:00 A.M. The air temperatures, at 4-hour intervals from 4:00 A.M. Friday, May 10th, to 4:00 A.M., Saturday, May 11th, were as follows: 57.5, 75.0, 98.0, 91.0, 72.0, 62.0, and 57.0° F., respectively. The relative humidities for the same hours were: 62, 32, 13, 35, 56, 86, and 82 per cent., respec-

tively. Evaporation from a black shallow pan (water 1 cm. in depth and 1,000 sq. cm. in area) evaporimeter was as follows: 4–8 A.M., 79 gm.; 8 A.M.–noon, 412 gm.; noon–4 P.M., 547 gm.; 4 P.M.–8 P.M., 149 gm.; 8 P.M.–midnight, 8 gm.; and, midnight to 4 A.M., 6 gm.

Results

Growth

A close-up view of the barley plants preceding harvest is shown in figure 1.

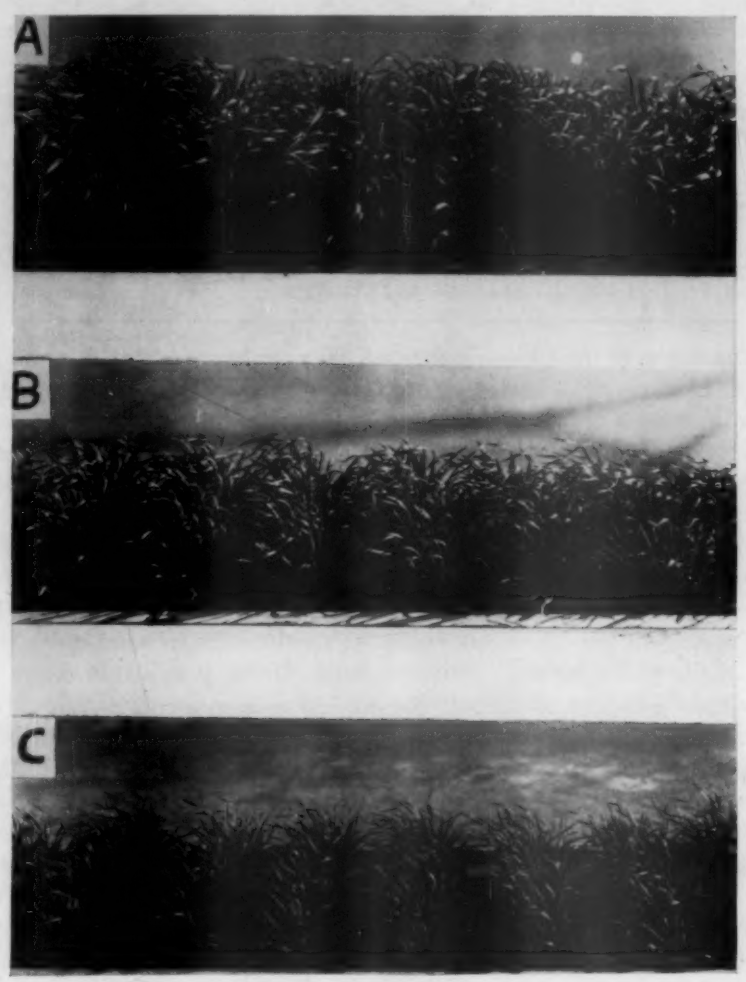

FIG. 1. Close-up view of barley plants on day preceeding harvest. A. Control. B. 100 m.e. of chloride per liter. C. 200 m.e. of sulphate per liter.

The march of dry weight of tops (based on 30-plant samples) through the 24-hour cycle is shown for each of the three treatments in figure 2, and the data are summarized in table II. By analysis of variance there is a highly significant difference between the weights of the plants in each of the three treatments. Growth was depressed more by 200 m.e. per liter of sulphate than by 100 m.e. per liter of chloride, which is in accord with the toxicity of the two ions found in earlier investigations with barley (9, table III).

TABLE II

EFFECT OF TREATMENT ON DRY WEIGHT AND MOISTURE CONTENT OF BARLEY SHOOTS

TREATMENT	FRESH WEIGHT	DRY WEIGHT	MOISTURE	ON RELATIVE BASIS		PERCENTAGE REDUCTION IN WEIGHT	
				FRESH WEIGHT	DRY WEIGHT	FRESH WEIGHT BASIS	DRY WEIGHT BASIS
	gm.	*gm.*	%			%	%
Control	6,396	598	90.6	100	100	0.0	0.0
100–Cl	2,778	367	86.8	43	61	57.0	39.0
200–SO_4	1,817	259	85.7	28	43	72.0	57.0

The slopes of the curves in figure 2 show an hour-to-hour increase in the weight of the control barley shoots throughout the 24-hour cycle, but little or no increase is shown in the weight of the chloride or sulphate shoots after noon. This finding could mean either that carbohydrates were only slowly synthesized in the salt plants after noon or that most of the photosynthetic product was translocated to the roots.

The continued weight increase of the control plants throughout the day indicates a more rapid synthesis of carbohydrates in these than in the salt plants. The lower sugar (fig. 5) and starch concentrations (fig. 6) in the control than in the salt shoots indicates, furthermore, a more rapid conversion of the photosynthetic product of the control plants into tissue structures than occurred in the salt plants. A greater accumulation of carbohydrates in the *roots*, as well as in the shoots, of the salt plants is implied by the fact that the control barley plants cropped at 4 A.M., May 10, died without developing new shoots from the stubble, whereas new shoots were developed from the stubble of all other croppings (see in this connection the carbohydrate data of figs. 5 and 6). The fact that shoots did develop from the stubble of the control plants cropped at 4 A.M. on the second morning (May 11) indicates that the slightly higher concentration of carbohydrates found in these plants on the second morning was above the minimum required for continued growth of shoots. Evidence of a higher proportionate translocation of carbohydrates to the roots of cereals grown on saline

than on non-saline substrates has been provided elsewhere (9) by the finding that the weight of roots of wheat in high-chloride solutions and corn in high-chloride and high-sulphate solutions was markedly greater than that found for corresponding control plants.

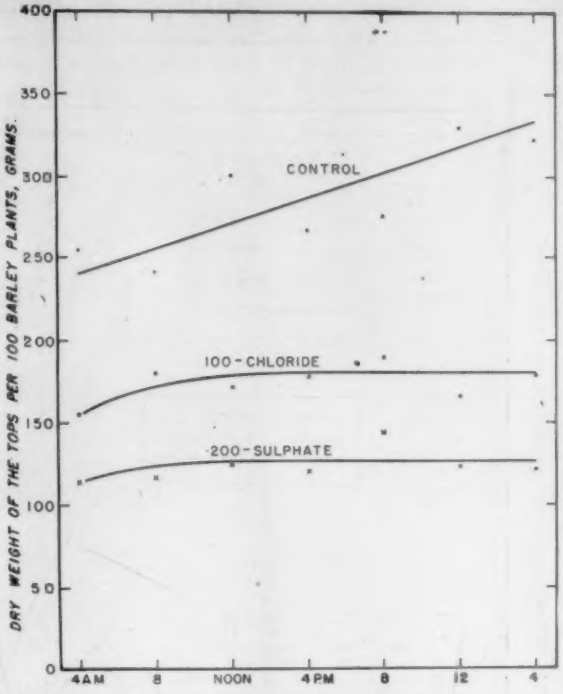

FIG. 2. Dry weight of tops per 100 barley plants at successive sampling hours.

MOISTURE CONTENT

The barley plants on the chloride substrate maintained a significantly lower average moisture content in their shoots than did the control plants and the sulphate plants operated at a significantly lower moisture level than the chloride plants (fig. 3). The differences found during the night hours, when evaporation was negligible, were only slightly more marked during the late afternoon when evaporation was high. There was thus a basic change in the tissue hydration characteristics of barley grown on saline substrates that is not related to evaporation rates. In another work (9), the succulence of barley as measured by the amount of sap that could be expressed from frozen tissue under uniform pressures was found to decrease sharply as the amount of either chloride or sulphate salt in the substrate was increased. No such effect was shown by milo, alfalfa, cotton, tomato, or sugar beet. In unpublished results SOKOLOFF and EATON found that dried and ground bar-

ley shoots grown on a sodium chloride substrate swelled and formed a nearly-impermeable mass when leached with water on a BÜCHNER funnel, whereas control and high-calcium barley plant material was easily extracted.

The decreasing moisture percentages through the three sets of plants (control—chloride—sulphate) are associated (fig. 4) with decreasing con-

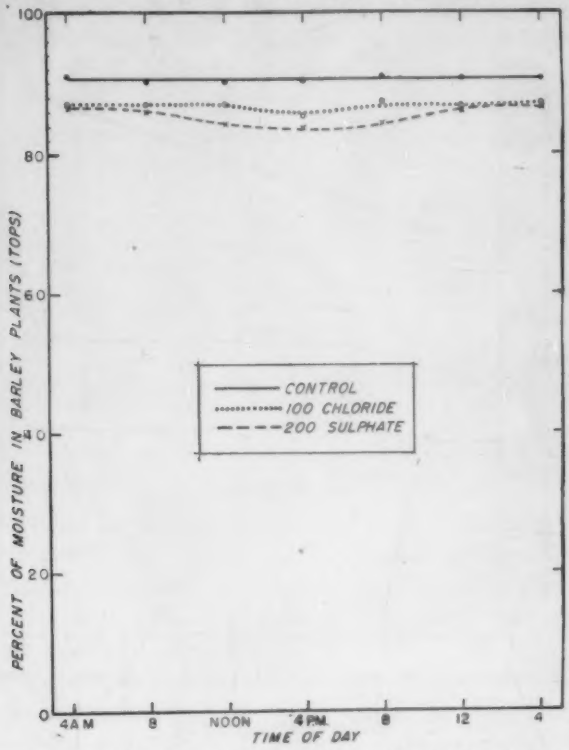

FIG. 3. Percentage of moisture in the tops of barley plants as related to treatment and time of day.

centrations of calcium, unchanged magnesium, sharply increasing sodium, decreasing potassium, and higher sucrose and starch concentrations in the shoots. From such diverse associations it would be difficult to assign a causal relationship to any one constituent. This is particularly true when it is recalled that GREGORY and SEN (11) reported "progressive potassium starvation leads to continuous increase in water content (in barley), while nitrogen deficiency has the reverse effect."

SALT ACCUMULATION

Little published evidence exists on the relationship between salt accumulation in plants and transpiration rates and yet in terms of salt toxicity and

the broad differences in climatic conditions found throughout irrigated regions this question can be regarded as basic. MUENSCHER (**16**), in general agreement with the findings of numerous earlier investigators who in nearly all instances confined their laboratory measurements to the determination of total ash, found that the ash of barley expressed in percentage of total dry weight of the entire plants varied but slightly regardless of whether the plants were grown under conditions of high or low transpiration and irre-

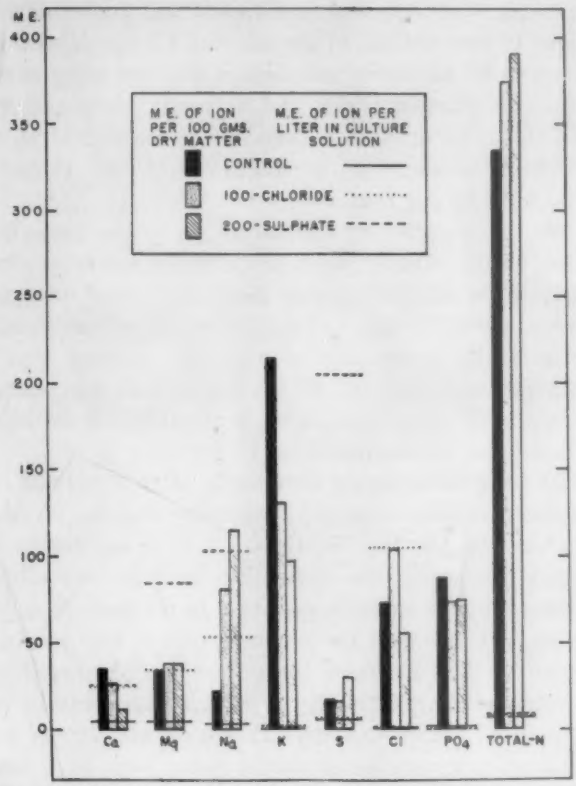

FIG. 4. The chemical composition of 5-week-old barley plants grown in base nutrient, in high chloride, and in high sulphate solutions.

spective of how transpiration was reduced. HOAGLAND and BROYER[2] on the other hand have found higher concentrations of sodium and chloride ions in the expressed sap of barley plants grown in a dry chamber than in that of plants grown in a humid chamber. They also report that the bromide ion is taken up from culture solutions and moved into roots, stems, and leaves

[2] HOAGLAND, D. R., and BROYER, T. C. Unpublished data presented at the 22nd annual meeting of the Pacific Division of the A.A.A.S., San Diego, California, June 21, 1938.

of squash and cotton more rapidly under the influence of light and humidity conditions conducive to high transpiration rates than under those conducive to low transpiration rates. Respiration rates and the availability of carbohydrates (**14**) as well as the oxygen supply to inner and outer root tissues (**5**) are now indicated as being factors that may markedly affect salt accumulation in the shoots of plants and are accordingly relevant considerations.

It seemed probable in advance of this study that if major relations existed between cyclic environmental factors and salt accumulation, the effect would be evident in comparisons of the concentrations found in this remarkably uniform series of plants as sampled in the late afternoon under conditions of high transpiration rates and at night when transpiration was negligible. In this connection it is desirable to recall that on the day covered by the samples the temperature rose to nearly 100° F. and the relative humidity dropped to 13 per cent.

FONDER (**10**) has reported on the variations in the amounts of calcium and magnesium of the alfalfa plant at different hours of the day. The plants were grown on Hillsdale sandy loam, and they were sampled at 4 A.M., 8 A.M., noon, 4 P.M., 8 P.M., and midnight. The leaves and stems were analyzed separately for percentage of moisture, calcium, and magnesium. He reported variations with time of day in the calcium, magnesium, and moisture contents in the stems and leaves, and variations in the calcium and magnesium contents of the expressed sap.

In this study separate analyses were made of each harvest of barley (as cropped in duplicate at succeeding 4-hour periods over the 24-hour cycle) for the following elements: Ca, Mg, Na, K, S, Cl, PO_4, and total nitrogen. As shown in table III, wherein the individual analyses are all reported, no evidence indicates that the extreme variation in transpiration rates over the 24-hour cycle has any effect on the accumulation of any ion in the tops of these barley plants. Furthermore, the period of most rapid carbohydrate accumulation coincided in part with the most rapid influx of water. Over this period, as pointed out by CANNON (**2**), the oxygen supply both from the substrate and from photosynthesis should have been at a maximum, yet there is no evidence that salt accumulation was thereby augmented. Accordingly, the conclusion would follow that neither oxygen nor labile respiratory materials fell at any time below the concentrations required for maximum salt absorption and retention in the shoot. Under the conditions of this experiment, the accumulation of inorganic constituents was affected only by the concentrations of the substrate. The extent of accumulation of a particular ion (fig. 2) is not dependent alone upon its concentration in the substrate; but instead, as has been extensively observed by other investigators, is dependent as well upon the uptake of other ions and their effects on the plant.

TABLE III

INORGANIC COMPOSITION OF 5-WEEK-OLD BARLEY PLANTS AT FOUR-HOUR INTERVALS IN A 24-HOUR CYCLE, AND THE COMPOSITION OF THE SUBSTRATE SOLUTIONS

TREATMENT: HOUR OF DAY	MILLIEQUIVALENTS PER 100 GRAMS OF DRY MATTER							
	Ca	Mg	Na	K	S*	Cl	PO_4	TOTAL NITROGEN*
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
Control plants								
4:00 A.M.	39 35	41 36	22 19	226 235	21 21	78 72	88 92	354 311
8:00 A.M.	36 36	36 36	22 19	218 216	19 13	66 63	88 83	358 352
Noon	34 31	31 31	26 20	238 216	19 13	71 66	83 80	339 355
4:00 P.M.	34 36	34 36	17 24	201 253	17 15	65 82	90 87	316 324
8:00 P.M.	37 39	36 38	23 24	239 254	21 18	76 80	88 78	323 319
Midnight	30 35	34 38	24 21	221 233	15 16	75 76	88 86	321 352
4:00 A.M.	30 34	32 37	18 24	220 223	15 15	66 73	89 85	311 360
Average	35	35	22	228	17	72	86	335
100-chloride plants								
4:00 A.M.	29 28	42 40	74 85	126 132	16 17	108 105	77 73	382 394
8:00 A.M.	26 27	36 39	77 77	133 128	17 18	103 101	75 71	385 368
Noon	26 26	37 39	77 80	128 132	15 14	104 109	77 72	388 380
4:00 P.M.	26 25	38 38	74 74	129 130	16 13	99 101	73 69	377 372
8:00 P.M.	27 27	38 32	82 84	136 140	13 18	106 107	90 67	363 347
Midnight	25 26	39 39	86 84	119 132	17 16	93 106	75 63	375 370
4:00 A.M.	26 26	39 40	85 85	126 118	17 16	91 92	79 70	373 383
Average	26	38	80	129	16	102	74	376
200-sulphate plants								
4:00 A.M.	12 12	37 37	122 121	127 92	31 27	54 55	55 72	399 375
8:00 A.M.	10 12	36 36	114 119	90 99	26 26	51 54	76 82	409 370
Noon	11 11	28 36	110 115	94 84	25 30	59 52	74 76	389 413
4:00 P.M.	11 10	37 30	115 105	91 98	26 27	51 58	79 73	395 380
8:00 P.M.	10 10	43 37	110 111	97 93	26 26	56 56	66 82	371 380
Midnight	11 12	40 38	116 125	93 97	31 27	53 55	78 81	376 399
4:00 A.M.	12 11	41 40	118 120	99 95	27 33	54 52	79 81	406 403
Average	11	37	116	96	28	54	75	390

* Total nitrogen and sulphur in the plant tissue reported on the basis of 0.014 grams N and 0.016 grams S per milliequivalent.

Calcium accumulation in the barley shoots was depressed by the addition of 20 m.e./l. of calcium to the culture solution when accompanied by the addition of other salts in amounts sufficient to raise either the chloride level of the culture solution from 4 to 104 m.e./l. or the sulphate level from 4.6 to 204.6 m.e. per liter. The magnesium level in the plants was unaffected by raising the concentration of magnesium in the substrate from 5.4 to 35.4 m.e./l. in the chloride solution and to 85.4 m.e./l. in the sulphate solution.

The sodium levels in the barley shoots were more directly related to the concentration of sodium in the culture solutions, than were the levels of any other ion. The control solution contained 1.9 m.e./l. of sodium and the plants contained 21.6 m.e. per 100 grams of dry material. An increase of 50 m.e./l. of sodium added as sodium chloride to the culture solution resulted in an increase of 59 m.e. per 100 grams of dry matter in the shoot; the addition of 100 m.e./l. of sodium sulphate to the base nutrient raised the level of sodium in the plant 96 m.e. per 100 grams of dry matter.

With no change in the potassium level in the culture solutions the addition of calcium, magnesium, and sodium salts to the solution in amounts sufficient to raise chloride by 100 m.e./l. resulted in decreased potassium in the plant from 228 m.e. to 129 m.e. per 100 grams of dry matter. With the addition of 200 m.e./l. of sulphate salts to the base nutrient the potassium level in the plant dropped to 96 m.e. per 100 grams of dry matter. The loss in potassium in the plant material may be related to sodium uptake, in the case of barley, but it is to be noted that the loss in potassium is not proportional, in the three sets of plants, to the gain in sodium.

Adding 200 m.e./l. of sulphate ion to the nutrient solution increased the sulphate level in the plant from 17.0 to 27.5 m.e. per 100 grams of dry matter.

With 4 m.e./l. of chloride in the culture solution the control plants accumulated 72 m.e. per 100 grams of dry matter. Adding 100 m.e./l. of chloride to the nutrient solution increased the chloride in the plant to 102 m.e. per 100 grams of dry matter. The addition of sulphate to the nutrient solution reduced chloride accumulation substantially but the addition of chloride was without effect on sulphate accumulation.

Nitrogen and phosphate accumulation was little affected by the addition of the chloride and sulphate salts to the base nutrient, notwithstanding marked changes in the concentration of other ions in the plant. The presence of the slightly larger quantities of nitrogen in the chloride and sulphate plants than in the control plants may indicate accumulation of unutilized nitrate ion associated with depressed growth but the minor reduction in phosphate with the salt additions remains unaccounted for.

In an earlier study (9) that involved analyses of the expressed saps of milo, alfalfa, cotton, tomato, and sugar beet grown together on substrates

with added chloride and sulphate salts, the accumulation responses of the different plants were found to be diverse. The present data on barley serve to emphasize further that great specificity exists among the plants in their accumulation characteristics and inter-ionic effects. These results from barley plants are especially noteworthy in the fact that sodium accumulation apparently depressed potassium accumulation but as is characteristic of many plants the accumulation ratio of potassium in the plant to that in the substrate is far higher than the sodium ratio. Barley, by these data, accumulates substantially more sodium than many other plants that have been studied (**4, 9, 19**). The 50-fold increase in the sodium concentration in the sulphate substrate brought about only a 5-fold increase in the sodium concentration in the plant.

DELEANO and GOTTERBARN (**6**) working with barley concluded that there is "antagonism" among the elements, calcium, magnesium, and potassium.

CARBOHYDRATES

The cyclic accumulation of carbohydrates in plant tissues has been extensively investigated but only a few citations will be made to this literature for the reason that in the present work the point of primary concern is that having to do with the effect of saline substrates on carbohydrate accumulation.

SUGARS.—PUHR and HUME (**17**) found that the maximal amount of sucrose and total sugar in the leaves of corn occurred between 1 and 4 P.M. MILLER (**15**) working with the leaves of corn and sorghums obtained maxima varying between noon and 5 P.M. CLEMENTS (**3**) observed maximal total sugar concentrations in the leaves of sunflower, potato, and soybean varying from 2 to 5 P.M. In the present experiment, for all treatments, the maximal concentration of sucrose and total sugar occurred in the samples taken at 4 P.M.

As shown in figure 5 and in table IV, the concentration of reducing sugars in fresh barley shoots was not materially affected by a differential accumulation of calcium, sodium, or potassium in the plant tissue nor by differences in the chloride and sulphate concentrations of the plant material. MILLER (**15**), whose findings are in accord with those of PUHR and HUME (**17**) on corn, reported that in corn and sorghum leaves the reducing sugars, as a rule, showed very little increase during the day and that the amount present at the different periods of the day was very irregular.

The concentration of the sucrose fraction was markedly affected by the salt treatments. Sucrose was substantially higher at nearly all hours of the day in the chloride plants than in the control plants and again higher in the sulphate plants than in the chloride plants.

The average concentrations (milligrams of sugar per 10-gram fresh plant

material) of total sugar in the three sets of plants (control, chloride, and sulphate) over the 24-hour period were 44.4, 58.4, and 67.9 mg., respectively. The 4 P.M. (maximal) values were 65.1, 97.2, and 106.6 mg., respectively.

The accumulation of sugars in the fresh tissue of the barley shoots in the three treatments stands in the reverse order of growth. It is thus fully evident, in the case of barley, that salt accumulation does not reduce the concentration of sugars and, in turn, that reductions in available sugars are

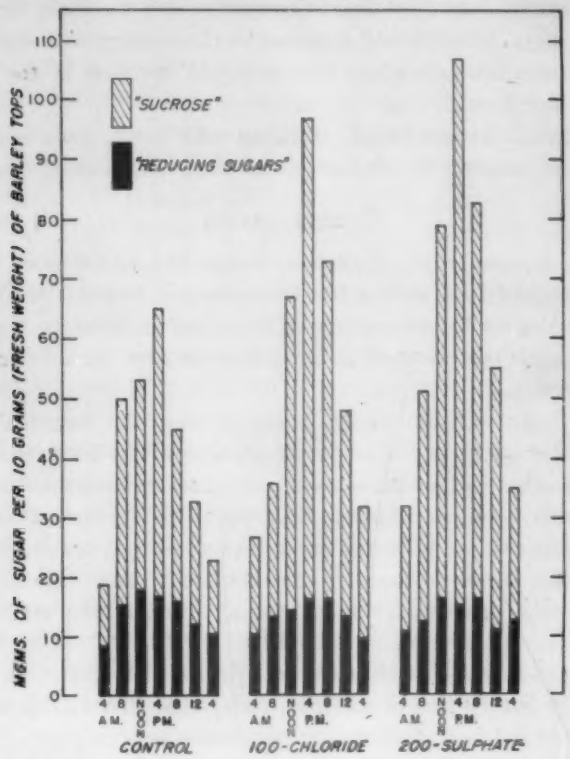

FIG. 5. The concentration of sugars in the shoots of 5-week-old barley plants, as affected by treatment and time of day.

not a direct or intermediate causal factor that will account for the reduction in growth on the saline substrates. The failure of the chloride and sulphate plants to utilize accumulated sugar and starch (see next section) in cell elaboration as rapidly as the control plants cannot be attributed to nitrogen or phosphorus deficiency since the levels of these elements differed little in the three sets of plants. The possibility remains that the depression in potassium concentration by the salt additions was responsible for curtailed

growth, but against this hypothesis is the fact that it would be difficult to argue insufficient potassium in plants containing 96 or 129 m.e. per 100 grams of dry matter. Furthermore, it has been shown elsewhere (**9**) that potassium depression does not occur in all plants with sodium accumulation. The fact of depressed growth and carbohydrate accumulation might on the barley evidence be attributed directly to sodium; but again, sodium accumulation does not always occur. Milo, for example, accumulates only a negligible amount of sodium and yet it is injured by salt concentrations such as those here used (**9**). The writers would hesitate to argue that the mechanism of salt injury is identical in different species; for it is highly hazardous to expound a general explanation on the basis of biochemical changes found to take place in some one plant.

TABLE IV

MILLIGRAMS OF SUGAR PER 10 GRAMS (FRESH WEIGHT) OF 5-WEEK-OLD BARLEY SHOOTS

TREATMENT: HOUR OF DAY	SUCROSE			REDUCING SUGARS			TOTAL SUGARS		
	EAST* ROW	WEST ROW	AVERAGE	EAST ROW	WEST ROW	AVERAGE	EAST ROW	WEST ROW	AVERAGE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Control:									
4 A.M.	10.3	11.1	10.7	8.7	8.5	8.6	19.0	19.6	19.3
8 A.M.	27.1	41.3	34.2	14.5	16.7	15.6	41.6	58.0	49.8
Noon	27.6	42.3	34.9	18.0	17.7	17.9	45.6	60.0	52.8
4 P.M.	56.0	40.5	48.2	16.0	17.7	16.9	72.0	58.2	65.1
8 P.M.	33.4	23.7	28.5	16.6	15.7	16.2	50.0	39.4	44.7
Midnight	17.1	23.5	20.3	13.1	11.5	12.3	30.2	35.0	32.6
4 A.M.	13.2	12.0	12.6	10.2	11.0	10.6	23.4	23.0	23.2
100-chloride									
4 A.M.	16.1	16.9	16.5	9.7	11.5	10.6	25.8	28.4	27.1
8 A.M.	20.4	23.8	22.1	13.0	14.2	13.6	33.4	38.0	35.7
Noon	52.8	52.0	52.4	16.2	13.0	14.6	69.0	65.0	67.0
4 P.M.	82.8	79.2	80.6	17.0	16.0	16.6	99.2	95.2	97.2
8 P.M.	58.6	55.0	56.8	17.0	16.0	16.5	75.6	71.0	73.3
Midnight	33.9	35.6	34.7	12.1	14.6	13.4	46.0	50.2	48.1
4 A.M.	22.8	21.4	22.1	9.0	10.0	9.5	31.8	31.4	31.6
200-sulphate									
4 A.M.	20.2	22.5	21.3	11.0	10.7	10.9	31.2	33.2	32.2
8 A.M.	38.3	37.7	38.0	11.7	13.3	12.5	50.0	51.0	50.5
Noon	56.0	69.5	62.6	17.0	15.5	16.4	73.0	85.0	79.0
4 P.M.	93.0	89.3	91.1	14.2	16.7	15.5	107.2	106.0	106.6
8 P.M.	71.4	61.4	66.4	14.0	19.8	16.9	85.4	81.2	83.3
Midnight	43.6	42.9	43.2	11.0	11.7	11.4	54.6	54.6	54.6
4 A.M.	24.7	20.4	22.5	12.5	13.0	12.8	37.2	33.4	35.3

* "East" and "west" refer to the duplicate rows that were harvested at each sampling period—one row of which was in the eastern half of the bed, and one row of which was in the western half of the bed.

STARCH.—The effect of substrate on the starch percentage was found to be marked during the late hours of the day and the differences between the

treatments (fig. 6) were statistically significant. The 4 to 8 P.M. maxima for the control, chloride, and sulphate plants were 1.00, 1.30, and 1.55 per cent., respectively.

The fact that the higher starch levels in salt plants are found to be associated with higher sugar levels on the fresh weight basis is regarded as indicating that polymerization is directly related to sugar concentrations in leaf sap.

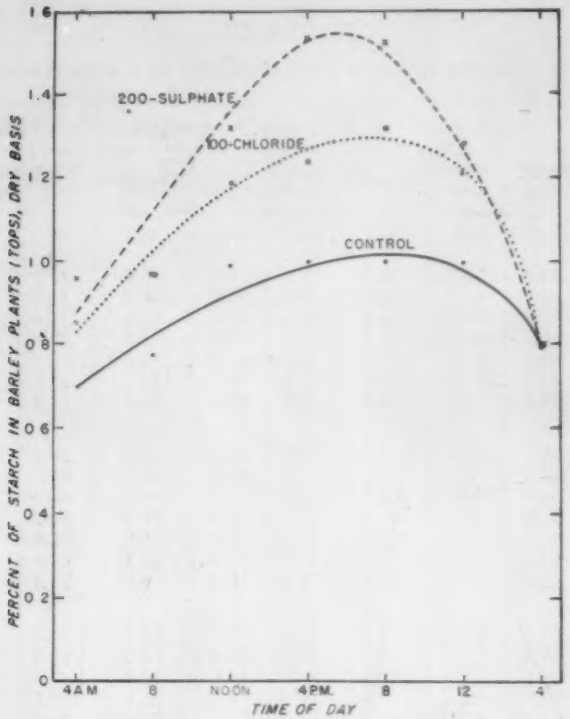

FIG. 6. Percentage of starch (dry weight basis) in the tops of barley plants.

That the enzyme system concerned with starch formation and starch hydrolysis was not impaired or inactivated by salt accumulation is clearly indicated by the rise and fall in starch accumulation. There is no clue in the starch cycle as to a mechanism of salt injury to plants.

Summary

1. With the purpose of determining (a) the relation between cyclic variations in transpiration rates on the accumulation of inorganic constituents in barley and (b) the effect of saline substrates on the accumulation of carbohydrates, barley plants were grown in sand cultures supplied with a base nutrient, base nutrient plus 100 m.e./l. of chloride, and with the base

nutrient plus 200 m.e./l. of sulphate. The plants were sampled at 4-hour intervals over a 24-hour cycle and analyzed for mineral constituents and carbohydrates.

2. The relative weights of the control, 100-chloride, and 200-sulphate plants on a fresh weight basis were: 100, 43, and 28; and on the dry weight basis: 100, 61, and 43, respectively. The average moisture content of the three sets of plants was: 90.6, 86.8, and 85.7 per cent., respectively.

3. Under all treatments essentially uniform concentrations of calcium, magnesium, sodium, potassium, sulphate, chloride, phosphorus, and nitrogen in the dry matter were found over the 24-hour cycle in which the temperature rose to 98° F. and the relative humidity dropped to 13 per cent. at noon.

4. The addition of both chloride and sulphate salts (24-hour averages) reduced calcium concentrations relative to the control plants, left magnesium unchanged, caused a marked increase in sodium, a marked decrease in potassium, and had little effect on total nitrogen and phosphate concentrations.

5. The accumulation of chloride in the control plants was notably high (72 m.e. per 100 grams dry matter), and the addition of 100 m.e./l. of chloride to the substrate brought about a further uptake to 102 m.e. per 100 grams of dry matter.

6. The control plants contained 17 m.e. per 100 grams of dry matter of sulphur and the 200-sulphate plants contained 27.7 m.e. per 100 grams of dry matter.

7. Associated with the foregoing increased concentration of sulphur in the plant material (sulphate plants) there was a depression in the amount of chloride (72 to 54 m.e. per 100 grams dry matter), but there was no change in the content of sulphur (chloride plants) associated with chloride accumulation.

8. At 4 P.M. the concentrations of carbohydrates in the control, chloride, and sulphate plants were, respectively: reducing sugars—16.9, 16.6, and 15.5; sucrose—48.2, 80.6, and 91.1; total sugars—65.1, 97.2, and 106.6 milligrams per 10 grams fresh weight of tops. In terms of percentage of dry weight, the starch values were, respectively: 1.0, 1.2, and 1.5 per cent.

9. The accumulation of carbohydrates was thus associated with salt accumulation, indicating that the salts interfered with the utilization of carbohydrates in cellular elaboration rather than with photosynthetic activity.

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WEEKLY ABSORPTION OF NITRATE BY YOUNG, BEARING ORANGE TREES GROWING OUT OF DOORS IN SOLUTION CULTURES[1]

H. D. Chapman and E. R. Parker

(with three figures)

Introduction

As part of a nitrogen nutritional experiment with young, bearing Valencia orange trees growing out-of-doors in complete solution cultures, a continuous record of the weekly absorption of nitrate and water, together with a thermographic record of air and solution temperatures, has been made for a period of approximately three and one-third years. Although these data do not indicate, save by inference, what periods of the year are most vital as regards the nutrient supply of nitrogen, they do show clearly the seasons of maximum and minimum absorption and afford information of interest as bearing on the relation of nitrate absorption to temperature conditions, periods of root and top growth, and transpiration. Data concerning total leaf number and leaf area and observations on their seasonal fluctuations and relations are included in this report.

To the knowledge of the authors, no studies of like nature have been recorded for citrus, although with reference to the seasonal requirements of these trees, Cameron (**1**), Cameron and Appleman (**3, 4**), and Cameron, Appleman, and Bialoglowski (**6**), from analyses of leaves, flowers, and young fruit from field trees, have indicated the importance of the nitrogen supply within the tree prior to and during the period of blossoming, spring growth, and fruit setting. These investigators and, more recently, Martin (**10**), and Hilgeman, Smith, and Draper (**7**), working with grapefruit in Arizona, found a marked reduction in the nitrogen of old leaves at the time of the spring blossom and growth cycle. Cameron *et al.* have also shown that the nitrogen of both top and root bark undergoes a reduction at this time and that substantial quantities of nitrogen are removed from the tree with the abscissed blossoms and small fruit. Unpublished data and observations accumulated by the authors in connection with nutritional and field experiments are in harmony with these findings, and it is widely held that ample nitrogen should be available to the citrus tree in the spring.

Experimental procedure

The detailed record of weekly nitrate and water absorption by the young

[1] Paper no. 451, University of California Citrus Experiment Station, Riverside, California.

citrus trees and of air and solution temperatures was begun September 27, 1937 and continued until January 13, 1941.

Two budded Valencia orange trees (nos. 25, 26) were employed for this study. These trees had been transplanted from the field nursery to solution cultures in May, 1936 (one year after budding) and had been grown in the solutions for a preliminary period of one year and five months.

The containers used in the preliminary period and during the first year in which records were kept were 100-liter vitrified tile sealed at the lower end and about three-quarters buried in the ground. Subsequently, the trees outgrew these tile containers and were transferred (November 14, 1938) to 600-liter concrete conduit provided with concrete bottoms. The concrete containers were painted inside and out with a water-emulsion type of

FIG. 1. Appearance of tree no. 25: A, in February, 1937, eight months before absorption record was begun; B, in January, 1941, at termination of experiment. Note the size of the foot scale at the right of each tree.

asphaltum. These, like the tile, were about three-quarters buried in the soil. With both the tile and concrete installations, concrete lids made in semicircular sections with semicircular holes at their adjoining centers, to accommodate the tree trunk, were used. These lids were provided with convenient holes to accommodate the aerator tube and to facilitate sampling and water additions (fig. 1).

The trees were grown in continuously aerated culture solutions of the following composition expressed in milliequivalents per liter: Calcium, 5.7; magnesium, 2.8; potassium, 1.0; sodium, 1.3; nitrate, 1.0; phosphate, 0.2; sulphate, 9.0; and chloride, 0.6. Manganese, boron, and aluminum in amounts to provide 0.5 p.p.m. of each, respectively, were added each time the

solutions were renewed and small amounts of ferrous sulphate were added daily.

Analyses for nitrate by the phenoldisulphonic-acid method were made on samples of solution taken every Monday morning, the solutions having been made up to a fixed volume by the addition of distilled water and thoroughly mixed by increasing the aeration rate before taking the sample. After the analysis, the nitrate concentration was adjusted by the addition of a mixed solution of calcium, magnesium, and potassium nitrate, in an amount to compensate for the week's absorption of nitrate. A second set of samples was then taken for analysis, to check on the resultant concentration of nitrate. No regular analyses were made for calcium, magnesium, or potassium, but the pH of the solutions was tested every 2 to 3 days and adjustments made with KOH or H_2SO_4 as required. Phosphate was determined weekly and adjustments made with KH_2PO_4.

A record of the amounts of distilled water added during the week to compensate for transpiration and the small loss from evaporation was also kept.

New solutions were provided every month. The slow growth of the trees, the large volume of solution employed, the weekly additions of nitrate to compensate for absorption, and the frequent pH and phosphate adjustments, together with complete renewal of the solutions every month, sufficed to maintain the concentration of the major ions reasonably constant.

At the time measurements of nitrate absorption were begun, the concentration of this constituent was reduced from 2.0 to 1.0 milliequivalent of NO_3 per liter. (Previous studies (**6**) with citrus seedlings in solution culture have shown that a nitrate concentration in the amount of 0.1 milliequivalent per liter is ample, provided the concentration is maintained and the solution stirred continuously.) On July 18, 1938, while the trees were still in the tile containers, it became necessary to increase the nitrate to 1.5 milliequivalents per liter in order to avoid the absorption of all of this constituent during the weekly period. Subsequently, on November 14, 1938, when the trees were transplanted to larger containers, the nitrate was reduced to 0.2 milliequivalent of NO_3 per liter; then, during the summer of 1939, the amount was gradually raised to 0.3 milliequivalent. On May 6, 1940, owing to the increased growth of the trees, it was necessary to raise the nitrate to 0.6 milliequivalent per liter. These adjustments were all made by decreasing or increasing the mixed nitrate solution additions to the culture solutions.

In four of the weekly periods during this experiment, namely, in the weeks ending July 18, August 1, and August 22, 1938, and May 15, 1939, nearly all of the nitrate present at the beginning of the week was absorbed by the end of the week. The remainder of the time, the amount of nitrate remaining in solution at the end of the week was above the minimum

requisite for good citrus growth. Save for the changes in concentration during the four weeks mentioned, when the nitrate was practically exhausted, and for the minor adjustment in nitrate concentration which became necessary as the trees grew, the nutrient conditions from week to week were essentially constant. Hence, the weekly quantities of nitrate absorbed may be regarded as primarily a reflection of the physiological activity of the tree.

At no time in the course of this experiment did these trees show detectable signs of malnutrition. The leaves were always deep green and the cycles of top growth coincided, in the main, with those of nearby field trees.

As stated, a continuous thermographic record of air and solution temperatures was kept, the recording instrument being housed in a suitably ventilated container adjacent to the cultures. The accuracy of the thermograph was checked at the outset, and maximum- and minimum-registering thermometers were installed in the thermograph house to check the accuracy of the air maxima and minima. The solution temperatures were also occasionally taken and compared with the thermograph reading. In general, agreement to within 1° F. was obtained throughout. The thermograph charts were changed at 9:00 A.M. on Monday mornings, and the mean weekly air and solution temperatures were calculated from planimeter measurements of the areas under the curves. The continuous stirring of the solution incident to aeration served to minimize inequalities of temperature within the solution.

The solution temperatures agreed closely with soil temperatures in nearby citrus orchards. The diurnal fluctuations of temperature in the solutions in the tile containers were of the same order of magnitude as those at the 12-inch soil depth. Those in the larger concrete containers were more like the fluctuations at the 24-inch soil depth. A series of comparative values are given in table I. Reference to this table shows that during some weeks the average temperature of the solution was somewhat lower and at other times higher than that of the soil at a depth of 24 inches. In view of the fact that there is a temperature gradient with depth in soils, it is evident that the temperature conditions under which these experimenal trees were grown were approximately similar to those which obtain in the field.

At periodic intervals during the three-and-one-third-year period, a count of all the leaves of each of the two trees was made. The leaves were graded into several size groups based on length, and counts of the number of leaves in each group were made each time. Having determined the area of leaves within each size group, it became possible to estimate total leaf area quite satisfactorily. Owing to new cycle growth on the one hand, and leaf senescence and abscission on the other, these counts are valid during the growing season for periods of only a few weeks before and after the leaf

TABLE I

COMPARISON OF MEAN WEEKLY TEMPERATURES OF SOLUTION CULTURE AND OF SOIL IN NEARBY CITRUS ORCHARD

DATE	MEAN WEEKLY TEMPERATURE	
WEEK ENDING	SOLUTION CULTURE	SOIL, AT DEPTH OF 24 INCHES
	°F.	°F.
1939		
Feb. 20	51.6	51.7
" 27	56.8	53.1
Mar. 6	55.0	50.1
" 13	57.0	56.4
June 5	74.0	69.5
" 12	77.0	71.2
" 19	77.0	72.2
July 3	81.5	73.5
" 31	84.0	78.1
Oct. 2	72.0	74.8
" 9	69.0	71.7
" 16	73.0	68.0
" 23	75.0	69.8
" 30	70.0	67.5
Dec. 4	59.0	61.2
" 11	62.0	61.3
" 18	60.0	61.3
" 25	58.5	60.0
1940		
Jan. 1	52.0	57.0
" 8	53.5	59.1
" 15	52.0	58.2
" 22	57.0	55.6
" 29	57.0	55.0
Average	64.52	63.32

count. During the first year (1938–1939), the fruit which set in April was picked early in June. In the two years of 1939–1940 and 1940–1941, however, all the fruit retained by the trees was allowed to ripen.

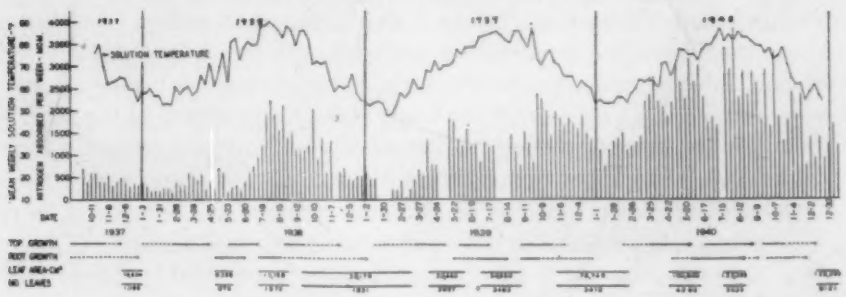

FIG. 2. The weekly absorption of nitrate nitrogen by a young Valencia orange tree (no. 25) growing out-of-doors in solution culture from September 27, 1937, to January 13, 1941.

From time to time it was necessary to spray the trees with an oil emulsion, with nicotine sulphate, and with tartar emetic to control red scale, aphids, and thrips, respectively.

The appearance of tree no. 25 in February, 1937 (eight months before the start of this experiment), and again in January, 1941, at the termination of the experiment, is shown in figure 1. The leaf count for this tree on the latter date was 5,121. This tree bore 36 pounds of fruit during the 1940–1941 season.

Results

In order that this paper may not be burdened with a large amount of tabular material, only the most pertinent data derived from this study are included.

The weekly absorption of nitrate by trees no. 25 and 26 from September 27, 1937 to January 13, 1941, together with the mean weekly temperatures of the culture solutions, are shown in figures 2 and 3 respectively. The mean air temperature, though averaging slightly lower than the mean solution temperatures, showed parallel weekly and seasonal changes. Since these data add nothing essential to the interpretation of the data given, they are omitted from the charts. The periods of top and root growth are shown by

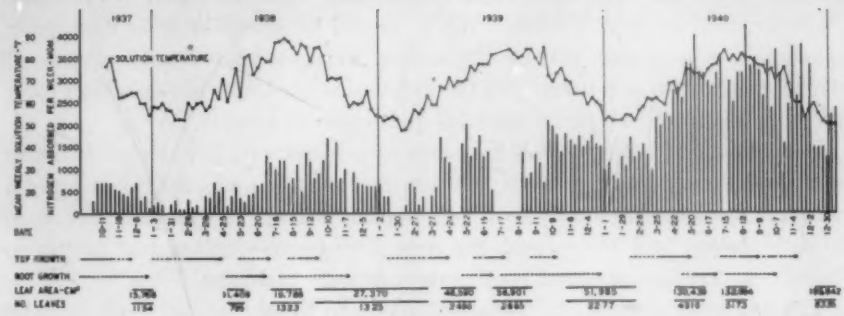

FIG. 3. The weekly absorption of nitrate nitrogen by a young Valencia orange tree (no. 26) growing out-of-doors in solution culture from September 27, 1937, to January 13, 1941.

means of horizontal arrows below the graphs. The solid portions of the arrows represent periods of more active growth; the broken portions, those of less active growth. Shown, also, by horizontal black lines below the graphs are the periods during which the leaf count and the determination of leaf area were reasonably accurate. The total leaf area, in square centimeters, and the leaf count for these periods are indicated immediately below the lines.

It is apparent that, under the climatic conditions prevailing in Riverside, California, nitrate absorption occurred throughout the year. As might be

expected, however, it differed greatly in rate at various seasons. For the three years of this experiment, the period of least absorption was in January and February. This was also the period of minimum solution and air temperatures. The period of greatest absorption and also of maximum solution and air temperatures was in the summer and fall. Although a broad correlation was thus obvious between the quantity of nitrate absorbed and solution temperatures, there were weeks and periods when the correlation was not close. The lowest mean solution temperature for any week in which nitrate absorption data were available was that of 50.2° F. for the week ending January 13, 1941. Substantial absorption of nitrate took place during this week and other weeks when the temperature was almost as low.

It is of interest to note that substantial absorption of nitrate occurred in September, October, November, and even in December in every year. While there was usually some top and root growth during this period, it was ordinarily very much less than that occurring in the spring and summer. The favorable solution and air temperatures which prevailed throughout these months in the particular years during which this experiment was conducted, were no doubt the controlling factors in this absorption.

From January until May of each year there was no root growth. During this period the roots assumed the characteristic light-brown to brown color of mature, healthy feeder roots. While, as will be shown later in this paper, periods of more intense nitrate absorption were associated with periods of active root growth, it is clear that the older suberized and non-growing roots were capable of absorbing substantial quantities of nitrate.

Beginning in March of each year and coincident with the period of active bloom and spring-cycle growth, nitrate absorption increased, even though there was at that time no sign of root development. It was not until most of the blossoms had fallen and the new spring-cycle leaves had begun to harden that the first signs of root growth became manifest.

The tendency toward alternation of root and top growth, though striking at this time, became less marked as the growing season progressed and was least prominent in the fall. In the fall a certain amount of root and top growth proceeded simultaneously.

Root and top growth in relation to nitrate and water absorption

In an experiment of this type with no control over climatic factors, it is difficult to determine the differential influence of many conditions, both climatic and physiological, on nitrate absorption. An indication, however, of the relative importance of root versus top growth, as affecting nitrate and water absorption, respectively, was observed in the spring and summer of 1938.

After the spring-blossom growth cycle of 1938, the two trees of this

experiment (nos. 25 and 26) got out of phase with one another in respect to root and top growth. Tree no. 25 began, on May 23, 1938, to send out a very profuse flush of new top growth accompanied by very little root growth; whereas new top growth on tree no. 26 was limited, but the root growth was abundant. The leaf count for these two trees on May 18, 1938, just before the onset of this differential behavior, was approximately the same, being 874 for tree no. 25, and 795 for tree no. 26. The amounts of water and nitrate absorbed during the several weeks preceding May 23 were also approximately the same for each tree. On May 30, however, as shown in the data of table II, differences in the relative absorption rate became

TABLE II

ABSORPTION OF NITRATE NITROGEN AND WATER IN RELATION TO ROOT AND TOP GROWTH OF TREES

Absorption period, 1938	Tree no. 25* (top growth profuse, root growth limited)†			Tree no. 26* (root growth profuse, top growth limited)†		
	Nitrogen absorbed	Water absorbed	Absorption ratio, nitrogen (mg.) / water (liter)	Nitrogen absorbed	Water absorbed	Absorption ratio, nitrogen (mg.) / water (liter)
	mg.	*liter*		*mg.*	*liter*	
May 9–16	600	6.47	92.7	600	6.80	88.2
May 16–23	198	.....‡	.....	350	.....‡	.....
May 23–30	280	8.92	31.4	250	10.95	22.8
May 30–June 6	320	7.52	42.5	420	9.14	45.9
June 6–13	200	.....‡	.....	450	.....‡	.....
June 13–20	400	10.64	37.6	630	7.91	79.6
June 20–27	600	14.80	40.5	680	9.97	68.2
June 27–July 4	750	15.24	49.2	1300	8.00	162.5
July 4–11	950	15.70	60.5	1150	10.00	115.0
May 23–July 11	3500	72.82	45.3	4880	55.97	79.1

* Leaf count May 18, 1938: tree no. 25, 874; tree no. 26, 795.
† Beginning week of May 23, 1938.
‡ Rain during this week.

manifest; tree no. 25 (with the heavy leaf growth) absorbed more water and less nitrate than tree no. 26, whereas the latter absorbed more nitrate and less water. These data provide a striking illustration of the differential influence of leaf growth versus root growth on water and nitrate absorption. In going over all of the water and nitrate absorption data for the two trees of this experiment, it became apparent that while in general large increases in water absorption were ordinarily accompanied by increases in nitrate intake there were, as illustrated in table II, many exceptions.

Another interesting, though by no means unexpected, observation was that in none of the weekly intervals during the course of this experiment were nitrate and water taken in by the plant in the same proportion as existed in solution. In all cases, nitrate was absorbed relatively more rapidly than the water. This is brought out by the data of table II, where the absorption ratio of milligrams nitrogen per liter of absorbed water varied in the experimental period covered from 22.8 to 162.5. During this period, nitrate nitrogen was adjusted at the beginning of each week to 14 p.p.m. It is evident that if nitrate and water were absorbed in the proportion existent in the solution, the absorption ratio should have been 14 or less (nitrate concentration gradually diminished during the week).

All of these observations merely constitute further evidence in a field which has been under investigation by HOAGLAND (**8, 9**) and others over a long period: different, though not necessarily disassociated, mechanisms are concerned with the intake of water on the one hand, and nutrients on the other.

LEAF COUNT AND LEAF AREA

Of interest aside from their use as indices of top growth in relation to nitrate absorption, are the data with respect to leaf area and leaf count at different times of the year.

The increase in leaf number and leaf area was not smoothly progressive. For example, the leaf count in May, 1938, was less than that in December, 1937; and in December, 1940, the count was less than in August, 1940. These fluctuations are of course the result of seasonal leaf fall which may exceed in amount the concomitant or ensuing new-cycle growth. CAMERON (**2**) has shown that this seasonal fluctuation is marked in the case of mature, bearing citrus trees in the field.

Of interest, also, is the fact that there was no constant relation between leaf count and total leaf area. It is a common observation that certain cycles of growth may consist, predominantly, of small leaves and others of large leaves. When senescence and abscission of a cycle of small leaves coincide with a new cycle of large leaves, an increase in leaf area occurs without great change in total leaf number. Such a condition was noted in the fall of 1938. The leaf count of tree no. 25 in July was 1,670, and the leaf area was 15,758 sq. cm. (fig. 2); in November, after the fall-cycle growth, the leaf count of this tree was 1,831 (a relatively small increase) but the leaf area had doubled to 32,179 sq. cm. The converse took place in the spring of 1939 when, owing to the emergence of a large number of small leaves and the abscission of older and larger leaves, the leaf count increased from 1,831 to 3,007, whereas the leaf area increased only from 32,179 to 32,493 sq. cm. It is clear from these data that the relation of leaf number to leaf area is not

constant and that any attempt to correlate physiological activity with leaf number must take account of the sizes as well as of the age of the leaves.

Discussion

With reference to the seasonal nitrogen requirements of citrus, the data of this paper are suggestive. General experience and the experimental work of others on citrus orchard trees (**1, 3, 4, 5, 7, 10**) indicate, as mentioned at the outset, the importance of nitrogen reserves within the tree prior to and during the spring growth and blossom cycle. In California, the first signs of spring growth on citrus occasionally appear in January and usually in February although the maximum development of flowers and leaves does not ordinarily take place until late March and early April. A heavy draft upon the reserve nitrogen and carbohydrate of the tree occurs during this period. Nitrate absorption, however, is at its minimum in the immediately preceding winter months. This suggests that the major accumulation of the nitrogen reserves necessary for vigorous spring growth, blossoming, and fruit setting may take place in the preceding fall and early winter when large amounts of nitrate are taken in by the tree, as shown by the data. It seems quite unlikely that the nitrate which begins to be absorbed in increasing amount at the time of the blossom peak is of consequence in-so-far as it affects the amount and character of the blossoms and leaves; but it doubtless becomes of great value to the young developing fruit.

Summary

A record of the weekly absorption of nitrate by two young, bearing Valencia orange trees growing out of doors in complete nutrient solutions for a continuous period of over three years has been made.

The period of least absorption in each of the three years was during January and February. The period of maximum absorption was that of late spring, summer, and early fall. These periods of varying absorption, as is to be expected, were related to solution and air temperatures. The week to week absorption varied widely at times, however, even when temperature conditions were essentially constant.

The relation between the quantities of nitrate and of water absorbed was quite variable from week to week. During one period when the two trees used in these experiments were out of phase with one another in respect to root and top growth, it became apparent that nitrate absorption was more closely related to periods of active root growth; on the other hand, water absorption was more closely related to cycles of active top growth or, more specifically, to the physiological activities associated with root and top growth. Under the conditions of low nitrate concentration prevailing throughout the experimental period, nitrate was absorbed relatively more rapidly than water, although the degree of selectivity varied quite widely

from time to time. While periods of rapid nitrate absorption were usually associated with periods of active root growth, substantial amounts of the constituent were taken in by roots which had become brownish colored and partially suberized.

From January to May in every year no new root growth occurred, the spring blossom and leaf cycle having completely emerged before the first signs of new root growth became manifest.

Most of the cultural operations and nitrate analyses incident to this experiment were taken care of by DAVID RAYNER and W. ROSSKOPF, Laboratory Assistants in the Experiment Station. The authors wish to acknowledge with thanks, this assistance.

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SOME EFFECTS OF RADIANT ENERGY IN RELATION TO ETIOLATION[1,2]

JOSEPH P. BIEBEL

(WITH THREE FIGURES)

Numerous investigators have worked on the problem of determining the effect of small quantities of visible radiant energy on seedlings and other plants with stored foods. The literature is extensive and goes back over one hundred years. MACDOUGAL (**6**) has thoroughly reviewed the work before 1900 while TRUMPF (**9**) and BURKHOLDER (**1**) have reviewed this and much of the later work.

The work before 1900 established the fact that visible radiant energy alone could be considered capable of initiating the morphological differences resulting when seedlings and plants with storage organs received small amounts of energy but not enough to carry on significant amounts of photosynthesis. Since 1900 more quantitative investigations have been carried out by TRUMPF (**9**), PRIESTLY (**7**), WITHROW (**13**), and WENT (**11**).

The following experiments were an attempt to give further information on the quantitative responses of certain seedlings to irradiation, and to find out more about the internal mechanism causing these responses.

Procedure

All the work was carried on in an air-conditioned darkroom. Photosynthesis was kept to a negligible level, either by exposing the plants to very low intensities of irradiation, or by giving them short daily exposures to higher intensities. Under these sets of conditions little or no chlorophyll was formed and the plant had to exist on stored food.

The temperature was kept between 20° and 22° C. except in one experiment where it was purposely lowered to 1° C. for 0.5 hour each day. Relative humidity was kept between 67 and 73 per cent.

The incandescent tungsten filament lamp was used as the source of radiant energy. The radiant energy from the lamp was filtered through 11 cm. of water and four thicknesses of red cellophane. The transmission of the cellophane was measured with a spectrophotometer and four layers were found to completely absorb all visible radiant energy below wavelength 5,850 Å. and to transmit uniformly about 60 per cent. of all wavelengths of visible energy above 6,200 Å. The plants were thus irradiated by energy in the yellow, orange, and red regions of the visible spectrum plus the near

[1] Contributions from the Hull Botanical Laboratory 544.

[2] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

infra-red spectrum up to 11,000 Å. WITHROW's (13) work demonstrated that when red kidney bean seedlings were exposed to radiant energy in the near infra-red beyond 8,000 Å. the responses described later did not occur. This was confirmed in one experiment when a few kidney bean plants were exposed for one half hour daily to the radiant energy of a 300-watt lamp in a deep bowl reflector placed 50 cm. above the tops of the plants. The radiant energy was filtered through 11 cm. of water and a plate of Corning infra-red transmitting glass number 254. This glass filter absorbs all visible radiant energy and transmits an average of 20 per cent. of the infra-red energy from 8,000 Å. to 11,000 Å. After a week of such exposure the plants appeared similar to the checks which had never been irradiated. The effective spectrum was therefore from 5,850 Å. to something less than 8,000 Å. All figures of irradiance are given in terms of this band.

The irradiance, or the intensity of irradiation, was varied by the use of different wattages of lamps, by varying the distance of the lamp from the plant, and by the use of neutral screens of white cloth. Differences in color temperatures of the various wattages of lamps used were not considered significant because of the restricted spectrum. The energy in this spectral region was very effective in causing the response and its use eliminated consideration of responses such as phototropism, which are correlated with the shorter wavelengths of the visible spectrum.

Most of the experiments were carried out using *Phaseolus vulgaris* variety red kidney, as the test plant, but from time to time other plants were used. The plants used were *Pisum sativum* variety Alaska, *Zea mays*, *Xanthium pennsylvanicum*, *Helianthus annuus*, and *Vicia faba*. Not all of the plants used responded to irradiation to the same degree, but the same general response was obtained from all. The general nature of the response can be observed in figure 1.

Most of the experiments were terminated seven to nine days after the seedlings appeared above the surface of the gravel used as the nutrient solution medium and about 13 to 15 days after the seed was sown. At the end of eight days of irradiation the cotyledons were quite shrunken and were nearly depleted of reserve foods. All experiments were repeated at least once.

A number of different problems was investigated. The first problem studied was to find how bean seedlings responded to increasing quantities of radiant energy of the longer wavelengths of the visible spectrum. The quantity of energy could be increased both by increasing the irradiance and by lengthening the time of irradiation. Both methods were used and the relationship between them was studied when both were varied so that the quantity of energy was kept constant. When irradiance was increased the time of irradiation was decreased and *vice versa*.

The second problem was to study the kinetics of the mechanism of the response. By varying the rate of cycles of alternating irradiation and non-irradiation over different lots of plants, but keeping the total energy constant, it was possible to obtain some information on the nature of the reactions involved in the response of the plant. By this technique it was also possible to differentiate between the mechanism involved in chlorophyll formation and the mechanism involved in the morphological response of bean seedlings, though both occur simultaneously under most conditions. Lowering the temperature at the time of irradiation and using a high irradiance gave

FIG. 1. The effect of the quantity of radiant energy. Left to right, 50,000 ergs/cm.^{2}/sec. for 15 minutes daily; 500 ergs/cm.^{2}/sec. for 15 minutes daily; 5 ergs/cm.^{2}/sec. for 15 minutes daily; and non-irradiated.

information on the Q_{10} of the limiting reactions in the mechanism at the time of irradiation.

The third problem studied was to find out what pigment or pigment systems absorbed the visible radiant energy. Albino and normal maize seedlings were irradiated to see if chlorophyll or the carotenoid pigments were involved in the mechanism of the response.

The fourth problem studied was to find out if all parts of the plant were equally sensitive to visible radiant energy and to see whether other parts of the plant would respond if one part of the plant were irradiated. This was usually done by covering all but the part to be irradiated. In some

cases various parts of the plants were removed and the effect of irradiation on the remaining parts was studied.

The fifth problem studied was the possible correlative relationship between the decrease in hypocotyl elongation associated with irradiation and auxin concentration in the hypocotyl.

Results

Effect of varying quantities of incident radiant energy

An experiment was conducted to find the lowest irradiance that would still cause a perceptible response. When exposed to an irradiance of 0.05 erg/cm.^{2}/sec. applied continuously, red kidney bean, pea, and broad bean all showed clear responses.

High irradiances with continuous irradiation to obtain the maximum response were not used since a few preliminary experiments showed that irradiances sufficient for photosynthesis would be required. Accordingly most of the work was done on the basis of exposures of 30 minutes or less daily. Little or no chlorophyll was formed under these conditions and the plants responded about the same as they did to continuous irradiation. With very little chlorophyll present, photosynthesis was negligible.

TABLE I

Exposure time constant, irradiance increased

Treatment	No. plants per plot	Av. length hypocotyl	Av. length first internode	Av. length petiole	Av. length × width blade
		cm.	*cm.*	*cm.*	*sq. cm.*
Non-irradiated	33	29.0	15.7†	0.7	.....
5 H* 15 min. daily	34	24.8	18.1	3.1	.....
500 H 15 min. daily	22	21.9	14.7	6.2	.....
50,000 H 15 min. daily	13	15.7	10.0	4.3	.....

* H = ergs/cm.^{2}/sec.
† Elongation had not yet ceased.

Table I gives the data from an experiment in which the time of irradiation was kept constant at 15 minutes daily but the irradiance was increased 10,000 times in two steps from five ergs/cm.^{2}/sec. to 50,000 ergs/cm.^{2}/sec.

The bean seedlings gave definite responses up to the highest irradiance used. Those growing under the highest irradiance in the chambers were similar in form to the same age beans growing in the greenhouse in the winter, but were nearly devoid of chlorophyll.

As table I shows, hypocotyl length decreased as the irradiance increased. Internodes also decreased in length in the same manner. Petioles increased in length up to rather high irradiances but further increase caused a decrease in the length. The change in the appearance of the plants can be seen in figure 1. The change in morphological response with increase in irradiance was greater than if the response increased simply as the logarithm of the quantity of incident radiant energy. It appears that high irradiances were more effective per unit of energy than low irradiances.

The total quantity of radiant energy incident on the bean plants was varied in one experiment by keeping the irradiance constant at 50,000 ergs/cm.^{2}/sec. and varying the length of time the plants were exposed to this energy from one second daily up to 900 seconds daily. Table II shows the results of this experiment.

TABLE II

IRRADIANCE CONSTANT, EXPOSURE TIME INCREASED

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH × WIDTH BLADE
		cm.	*cm.*	*cm.*	*sq. cm.*
50,000 H 1 sec. daily	13	25.3	14.7	3.8	5.5
50,000 H 10 sec. daily ...	11	23.3	10.3	4.8	14.5
50,000 H 30 sec. daily ...	11	19.3	10.5	4.8	18.5
50,000 H 60 sec. daily ...	13	19.0	10.0	4.7	20.2
50,000 H 300 sec. daily	10	17.4	9.9	4.2	20.6
50,000 H 600 sec. daily	10	17.5	9.8	4.5	21.6
50,000 H 900 sec. daily	9	16.6	9.2	3.7	21.2

When the quantity of radiant energy was increased by lengthening the duration of the exposure, the plants given the greater amount of energy had shorter hypocotyls, shorter first internodes and longer petioles. Petiolar length decreased, however, when the total energy was increased above certain levels. Length and width of leaves increased rapidly with increases in energy at short exposures but increased throughout, as did all the other responses, even when the duration of exposure was long. These morphological responses increased less than if they increased as the logarithm of the quantity of incident radiant energy. This can be seen easily when length of hypocotyls is compared. There was a greater difference in the length of hypocotyls between the plants exposed 1 and 30 seconds than there was between those exposed 30 and 900 seconds.

QUANTITY OF RADIANT ENERGY PER DAY KEPT CONSTANT BY VARYING BOTH IRRADIANCE AND EXPOSURE TIME

As a result of several experiments, TRUMPF came to the conclusion that the quantitative response of bean seedlings to irradiation depended on the total daily quantity of radiant energy incident on the plant surface; that is, a low irradiance for a long time daily was as effective as a high irradiance for a short time daily, provided the total daily energy incident on the plant was the same.

Several experiments were run to repeat those results but using a far greater range of irradiances than had been used heretofore. In one experiment the irradiance was increased from 50 ergs/cm.^{2}/sec. to 50,000 ergs/cm.^{2}/sec. The radiant energy applied was 4,500,000 ergs/cm.^{2}/day. The results are given in table III.

TABLE III

TOTAL ENERGY CONSTANT, EXPOSURE AND IRRADIANCE VARIED

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH BLADE
		cm.	*cm.*	*cm.*	*cm.*
52 H continuously	32	20.0	12.6	5.9	3.5
500 H 150 min. daily	28	19.4	12.5	6.2	4.1
5,000 H 15 min. daily..	22	18.2	10.5	4.3	4.3
50,000 H 1.5 min. daily..	24	17.7	9.5	4.1	4.6

The response to irradiation increased with increase in irradiance even though exposure time was reduced accordingly to keep the daily quantity of radiant energy constant. Similar results were obtained when the daily quantity of radiant energy was one-tenth and also one-thousandth the amount used in table III. These results are in contradiction with those of TRUMPF but are confirmatory of the recent work of WENT.

In an attempt to measure how much more effective the high irradiances are than the low, several lots of bean seedlings were given different exposures at a high irradiance and then compared with a lot of seedlings that were given a lower irradiance but for a much longer time so that the total radiant energy was the same as the longest exposure of the high irradiance lots. The results of one such experiment are given in table IV. The starred treatments are those having the same total energy daily.

The bean seedlings irradiated at an irradiance of 50,000 ergs/cm.^{2}/sec. for 10 seconds daily received one-third the total daily quantity of energy of the low irradiance plot, yet these two lots of plants showed an overall

TABLE IV

MEASUREMENT OF GREATER EFFECTIVENESS OF HIGH IRRADIANCES

TREATMENT	No. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH × WIDTH BLADE
		cm.	*cm.*	*cm.*	*sq. cm.*
18 H* continuously	24	21.0	13.5	6.8	13.0
50,000 H 1 sec. daily	13	25.3	14.7	3.8	5.5
50,000 H 10 sec. daily	11	23.3	10.3	4.8	14.5
50,000 H 20 sec. daily	11	19.1	10.9	4.6	14.5
50,000 H* 30 sec. daily	11	19.3	10.5	4.8	18.5

* These two plots have the same total incident energy.

response that was very similar. Since the only measurement of the effect of the radiant energy available was the ultimate form of the plant, it was assumed therefore, that irradiation at the high irradiance was approximately three times as effective per unit of energy as the low irradiance.

A COMPARISON OF DAILY IRRADIATION AND IRRADIATION ONLY ONCE DURING THE EXPERIMENT

Kidney bean seedlings responded efficiently to radiant energy applied for a single short exposure daily. Experiments were carried out to see if a single exposure to radiant energy at different times in the life of the plants was as effective as short daily exposures.

One lot of bean seedlings was irradiated with an irradiance of 500 ergs/cm.^{2}/sec. for 1.25 hours daily for six days and for 30 minutes on the seventh day making a total of eight hours of irradiation. Another lot was

TABLE V

A SINGLE IRRADIATION COMPARED WITH DAILY IRRADIATION

TREATMENT	No. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. FRESH WEIGHT LEAVES
		cm.	*cm.*	*cm.*	*gm.*
500 H 1.25 hr. daily	18	20.4	11.7	4.7	0.37
500 H 8 hrs. second day	18	23.1	13.6	3.9	0.26
500 H 8 hrs. fourth day	18	24.7	13.5	3.0	0.22
500 H 8 hrs. fifth day	18	27.5	9.8	2.4	0.22

irradiated at the same irradiance for eight consecutive hours the second day after the seedlings emerged and not thereafter; another lot was irradiated under the same conditions but only on the fourth day after emergence; and still another lot on the fifth day after emergence. All plants received the same total quantity of radiant energy but at different times during the experiment. The results are given in table V.

Daily irradiation was more effective than any single irradiation any time during the experiment. In comparing the various single exposure treatments with one another it is well to remember that the plants irradiated first had more time to develop and show the response to irradiation while those irradiated later had already elongated much before being irradiated and irradiation could not alter this. These also had the least time to respond before the experiment ended. This also holds for the last one or two irradiations where the plants were irradiated daily.

Cycles of irradiation other than once every 24 hours

The length of time between irradiations very greatly influenced the amount of the response. Several experiments were conducted to find the response of bean seedlings to periods of irradiation both more frequent and less frequent than once every 24 hours.

A number of experiments were run and the number of times the plants were irradiated varied from 144 times daily to once every three days. In all of these experiments irradiance was not varied but the duration of each period of irradiation was lengthened or shortened as the frequency of the cycles was short or long so that the plants received the same total quantity of radiant energy by the end of the experiment regardless of the frequency of the periods. For example, one plant received 7.5 minutes of irradiation

TABLE VI

Cycles more than once every twenty-four hours

Treatment	No. plants per plot	Av. length hypocotyl	Av. length first internode	Av. length petiole	Av. length × width blade
		cm.	*cm.*	*cm.*	*sq. cm.*
6000 H 5 min. every 24 hours	57	22.9	11.4	4.8	25.5
6000 H 10 min. every 24 hours	69	21.5	11.1	4.6	32.0
6000 H 7.5 min. every 12 hours	123	20.3	10.8	4.5	35.2
6000 H 15 min. every 24 hours	92	20.9	11.3	5.1	33.6
6000 H 22.5 min. every 36 hours	90	21.9	12.1	5.5	32.0
6000 H 30 min. every 48 hours	125	22.4	12.1	5.1	31.4

every 12 hours and another plant received 15 minutes of irradiation every 24 hours. Table VI shows the results of one such experiment where the cycles were multiples of 12 hours. To measure quantitatively the effect, two plots were run with shorter exposures than 15 minutes daily. If certain cycles of exposure were less effective than one exposure daily, the difference could be measured by comparing them with the plants given a daily exposure of less than 15 minutes.

Table VI shows that irradiation twice daily was the most effective and that as the time between irradiations was increased the response decreased even though the total quantity of radiant energy incident on the plant was the same. Irradiation 10 minutes daily produced about the same response

TABLE VII

CYCLES MORE THAN ONCE EVERY TWENTY-FOUR HOURS

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. FRESH WEIGHT BLADE	CHLOROPHYLL PER GM. FRESH WEIGHT LEAF
		cm.	*cm.*	*cm.*	*gm.*	*mg.*
500 H 1.25 hours daily	42	22.0	12.6	5.9	0.31	0.25
500 H 0.5 min. 144 times daily	39	21.3	12.8	5.6	0.31	0.47
500 H 15 min. daily	25	24.4	12.8	5.1	0.25	0.10
500 H 6.25 seconds 144 times daily	40	21.6	13.6	5.7	0.27	0.26
Non-irradiated	39	30.0	11.0	0.8	0.10	0.01*

* Comparison was difficult and the figure is probably high.

as irradiation for 22.5 minutes every 36 hours even though the latter treatment had 50 per cent. more energy incident on the plants. Irradiation of between 5 and 10 minutes daily was as effective in causing the response as irradiation for 30 minutes every two days. That is, it was approximately twice as effective since about half the quantity of radiant energy was required to produce the same degree of response.

In another experiment one plot was given 144 exposures daily of 6.25 seconds each, making a total exposure of 15 minutes daily. Another plot was given a single exposure of just 15 minutes daily. The results are given in the first five columns of table VII.

The plants irradiated 144 times daily had the shortest hypocotyls, but in all other respects they had the characteristics of plants that had received less energy; that is, the internodes were longer, the petioles were shorter and

the leaves were smaller. It appeared that irradiation once every 10 minutes was less effective than irradiation once every 24 hours in many respects and more effective in some.

In another experiment, not given here in tabular form, an exposure of three times daily was compared with an exposure of once daily. The plants produced by these treatments were almost identical, indicating that they were of about equal effectiveness in producing morphological responses. The former were, however, somewhat greener.

Whenever irradiation was continued for more than 30 minutes daily at fairly high irradiances, or where plants were irradiated more than once daily, visible quantities of chlorophyll appeared in the leaves of kidney bean seedlings. The chlorophyll concentration in the leaves of plants irradiated many times daily was measured in one experiment. SHERTZ's (**8**) modification of WILLSTÄTTER and STOLL's method of extracting chlorophyll was used and GUTHRIE's (**2**) standard was made up for comparison purposes. The results were determined in a Klett colorimeter. Table VII, column six, shows the results of two separate series.

Irradiation 144 times daily at least doubled chlorophyll concentration in the plants so irradiated as compared with those irradiated once daily. This was true when they were irradiated at 2,250,000 ergs/cm.^{2}/day as well as when irradiated at 450,000 ergs/cm.^{2}/day. The fact that chlorophyll concentration increased when they were irradiated many times daily, whereas the morphological response of the plants varied in no very consistent direction is evidence that chlorophyll formation and morphological response of kidney beans to irradiation are different mechanisms having different kinetics.

PLANT PIGMENTS AND THE RESPONSE TO IRRADIATION

Before any photochemical reaction can take place radiant energy must be absorbed. Some preliminary work was done in an endeavor to find out what pigment or pigment system was concerned in the absorption of the visible radiant energy. One method of attacking this problem was to use albino seedlings. Strains of albino beans or other dicotyledonous plants were not available, but a number of albino strains of maize were available and one of these was used.

Figure 2 shows the results obtained. The seedling on the left of each pair was an albino, that on the right was a normal seedling containing chlorophyll from the same parentage. The albino seedlings were completely white, indicating that they were free of apparent quantities of carotenoids as well as of chlorophyll. Both the albino and the chlorophyllous seedlings responded alike to the irradiation. Large amounts of seed were not available and only one fourth of the seedlings were albino so that only a few plants were available for comparison, but the results were clear enough.

The albino plants could respond to irradiation and responded to the same degree as their chlorophyll-containing sibs. No extractions were made of the albino maize seedlings to find out whether there were pigments present that could not be seen by casual observation.

The results obtained here do not specifically exclude chlorophyll or related substances from consideration as the radiant energy absorbing pigments, but they do show that if these are the pigments that absorb the radiant energy and initiate the response, the quantity of energy that is absorbed by the pigment is very small compared to the quantity that is incident on the plant surface.

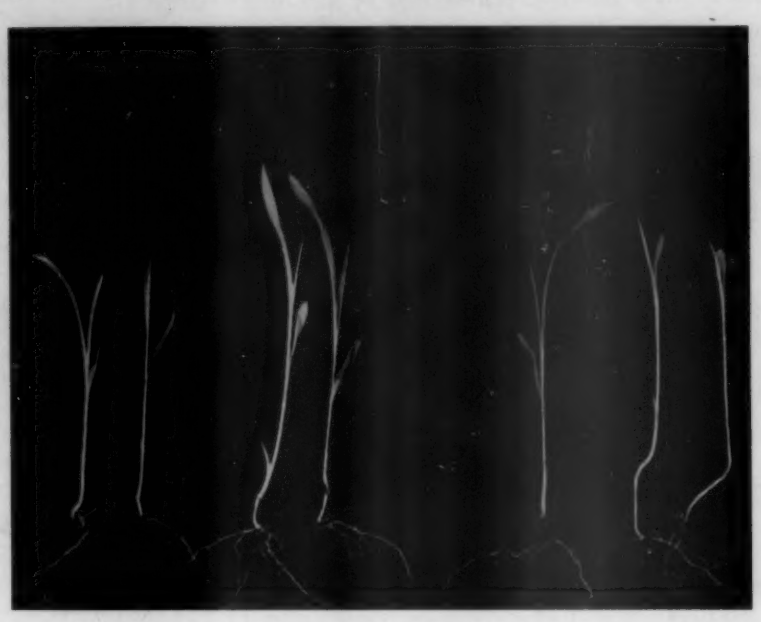

FIG. 2. Albino maize seedlings at the left of each pair except the third where the normal green seedling only is shown. The treatments per pair are left to right: 100 ergs/cm.^{2}/sec. continuously; 30 ergs/cm.^{2}/sec. continuously; 10 ergs/cm.^{2}/sec. continuously; and non-irradiated.

THE EFFECT OF TEMPERATURE AT THE TIME OF IRRADIATION

TRUMPF concluded that temperature had no effect on the morphological response to visible radiant energy. Several experiments were conducted to repeat and extend his work.

Several lots of bean seedlings were grown at 21° C. continuously until they began to appear above the surface of the gravel. One lot was irradiated daily before being kept at 1° C. for 0.5 hour; another was irradiated

daily at the time the plants were being kept at a temperature of 1° C. Still another lot was not irradiated at any time but was kept at the temperature of 1° C. for 0.5 hour daily just to see what effect the low temperature had on general growth. Two other lots were grown at 21° C. continuously, one was irradiated and the other not. The results are shown in table VIII.

TABLE VIII

EFFECT OF TEMPERATURE AT TIME OF IRRADIATION

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH × WIDTH BLADE
		cm.	*cm.*	*cm.*	*sq. cm.*
2500 H 15 min. daily during 0.5 hr. at 1° C.	33	18.2	11.4	4.7	23.5
2500 H 15 min. daily at 21° C.	24	18.1	11.3	4.8	23.0
2500 H 15 min. daily 21° C. continuously	35	20.3	11.8	5.8	25.5
Non-irradiated but 0.5 hr. at 1° C. daily	20	28.9	12.2	.....	.....
Non-irradiated 21° C. continuously	20	29.6	13.3	.....	.....

There were no significant differences either in the type or the magnitude of the response between the beans irradiated at 1° C. and those irradiated at 21° C. provided both lots were kept 0.5 hour at 1° C. some time daily. The low temperature had a stunting effect as can be seen by comparing plants that were maintained at a constant temperature of 21° C. with those that received 1° C. for 0.5 hour daily. This stunting was evident whether the plants were irradiated or not. The slowing down of growth by the short exposures to low temperature apparently had no direct bearing on the mechanism of the response to irradiation since it occurred on irradiated and non-irradiated plants alike, and since the response to irradiation was not altered when the plants were irradiated at 1° C. or at 21° C. provided both were given the temperature of 1° C. at some time. These results confirm TRUMPF'S work. The Q_{10} of the limiting reaction at the time the plants are being irradiated is unity or very close to unity.

LOCALIZATION OF THE RESPONSE TO IRRADIATION

Numerous experiments were conducted to see if any special part of the plant acted as a perceptor of the radiant energy or if each irradiated part of the plant acted separately as a perceptor of the irradiance. TRUMPF and WENT as a result of their work have come to the conclusion that each part of the plant acts separately.

Red kidney bean and pea variety Alaska were used as test plants. The first experiments were carried on at low irradiance, but the responses were small and inconclusive. Later experiments were carried on at higher irradiances, but extreme care had to be taken to prevent leakage of radiant energy.

In the early experiments the part of the plant that was not to be irradiated was wrapped in metal foil that was painted a dull black on both surfaces. This black wrapping was put on just before irradiation and removed after irradiation.

In later experiments with beans the part of the plant to be shielded from the radiant energy was slipped into a small cylindrical pasteboard carton with a removable lid. The slit in the lid was sealed up with black tape and the hole where the stem came through was sealed with modeling clay.

Most of the data from the localization experiments show that these plants had longer hypocotyls than those of the plants in the other experiments. This was unavoidable because the irradiation procedure could not be carried on until the epicotyl and leaves had developed so that they were large enough to work with. This was usually two or three days after the plants had emerged above the surface of the gravel. The hypocotyl had elongated a great deal by this time.

TABLE IX

LOCALIZATION, IRRADIANCE 5000 H 15 MINUTES DAILY FOR LAST FOUR DAYS

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. FRESH WEIGHT BLADE
		cm.	*cm.*	*cm.*	*gm.*
Non-irradiated	39	29.9	11.5	0.6	0.11
Hypocotyl only irradiated	8	30.2	12.5	0.8	0.13
Epicotyl only irradiated	9	28.7	13.2	1.3	0.14

TABLE X

LOCALIZATION, IRRADIANCE 5000 H 15 MINUTES DAILY FOR LAST THREE DAYS

TREATMENT	NO. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH × WIDTH BLADE
		cm.	*cm.*	*cm.*	*sq. cm.*
Entire epicotyl irradiated	15	31	12	2.9	8.1
Growing point only irradiated	12	31	12	2.5	7.0
Leaves only irradiated	13	31	13	1.6	4.9
Non-irradiated	29	32	13	.....	.....

Because of the individual treatment given each plant daily and because of the laborious technique involved, relatively few plants were used in any one experiment, but the experiments were repeated a number of times. Tables IX and X give the results of several typical experiments. Figure 3 illustrates table IX.

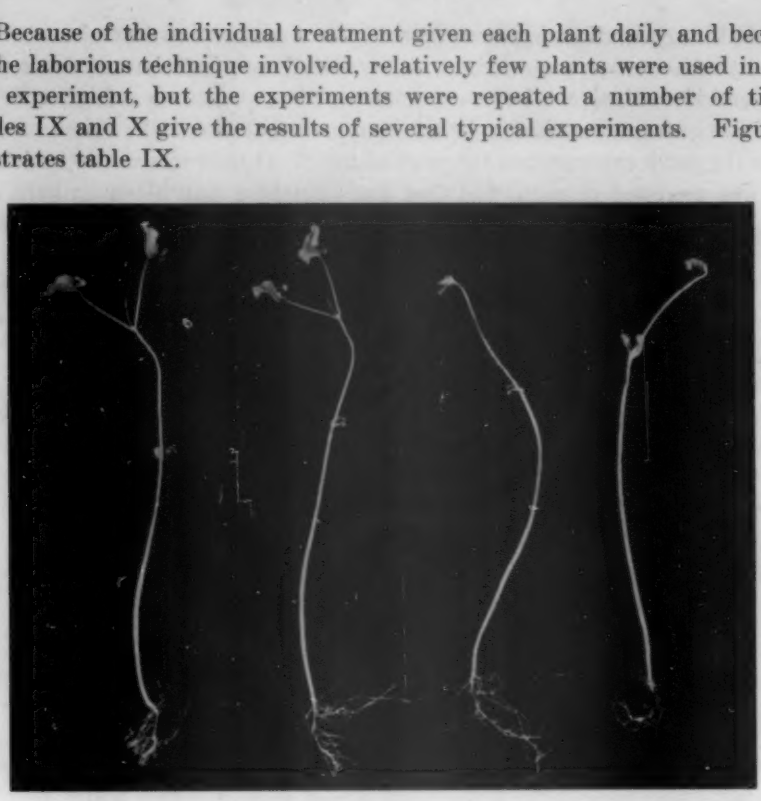

FIG. 3. Effect of localized irradiation of 5,000 ergs/cm.²/sec. for 15 minutes daily. Left to right, entire plant irradiated; epicotyl irradiated; hypocotyl irradiated; and non-irradiated.

It was apparent that the hypocotyl of kidney bean was very insensitive to radiant energy. The cotyledons and the first internode also were not very sensitive. Most of the sensitivity of the kidney bean seedling to long wavelength visible radiant energy was confined to the young leaves and the growing point. Both seemed to act as effective perceptors, but the growing point appeared to be the most effective perceptor since a marked response resulted when it was irradiated even though its surface area was far smaller than that of the expanding leaves.

No conclusive results were obtained to indicate that the response was completely localized, that is to say that only that part which was irradiated responded to the irradiation. Adjacent parts seemed to be affected to some degree. The various plant parts did differ in their sensitivity to irradiation.

The removal of some organs of a non-irradiated plant has been found in some cases (**3, 5**) to have caused the remaining organs to develop differently

than they otherwise would. To test this an experiment was conducted with kidney bean seedlings irradiated with 2,500 ergs/cm.²/sec. for 15 minutes daily. As soon as the plants appeared above the gravel the cotyledons of some plants were removed and the epicotyl of others. One day later, as soon as they had developed enough to work with, the leaves were removed from still another lot of seedlings. Check lots, both irradiated and non-irradiated, were left. The results are given in table XI.

TABLE XI

LOCALIZATION, ORGANS REMOVED, IRRADIANCE 2500 H 15 MINUTES DAILY

TREATMENT	No. PLANTS PER PLOT	AV. LENGTH HYPOCOTYL	AV. LENGTH FIRST INTERNODE	AV. LENGTH SECOND INTERNODE	AV. LENGTH PETIOLE	AV. LENGTH × WIDTH BLADE
		cm.	*cm.*	*cm.*	*cm.*	*sq. cm.*
Non-irradiated	23	21.0	16.7	.....	.....	.....
Entire plant irradiated	29	12.9	12.3	2.6	4.4	14.8
Leaves removed	18	12.6	14.0	3.3	.....	.....
Epicotyl removed	23	12.2	.....	.....	.....	.....
Cotyledons removed	13	10.9	8.2	0.2	1.3	2.9

The removal of an organ had three probable effects. Firstly, it relieved the remaining organs from the competition of the missing organ for the stored foods in the cotyledons, or in the case of the removal of the cotyledons it removed the main source of stored food itself. Secondly, perceptors of radiant energy were removed which had effects beyond the organ removed. Thirdly, the wounding may have caused unpredictable traumatic effects.

The removal of the cotyledons stunted the plant, but did not greatly change the qualitative response to irradiation. That is, the plant without cotyledons resembled the unmutilated plant, but was simply smaller in all dimensions.

Removal of the epicotyl seemed to have little effect on the development of the hypocotyl. It was slightly smaller than the checks. Apparently the hypocotyl and cotyledons were sufficiently sensitive to irradiation to produce a response similar to that obtained when the entire plant was irradiated. Non-irradiated plants in which the epicotyl was removed soon after emergence seemed to have about the same length hypocotyls as normal non-irradiated plants.

Removal of the developing leaves had little effect on the hypocotyl, but seemed to favor the development of the first and second internodes. This is the only evidence that the removal of a competing organ favored the de-

velopment of the remaining organs, but in this case a part was left behind which was very sensitive to radiant energy. The removal of the entire epicotyl removed the most effective perceptors. The removal of the leaves left the sensitive growing point intact. It is impossible to know whether the increased elongation of the first internode was simply a response to greater food supply, or was a response to an apparent lower effective irradiation brought about by a decrease in surface of perceptors of irradiation. The increased elongation of the second internode has usually been associated with more radiant energy, but again it may be due simply to a greater food supply.

Grafting experiments

A number of experiments were conducted in which a kidney bean seedling that had been irradiated for several days was grafted by the inarching technique to a non-irradiated bean seedling. The grafting operation was carried out in weak blue light since it was impossible to graft in total darkness. The radiant energy from a small wattage incandescent tungsten filament lamp was filtered through Corning glass number 511 which transmits the band 3,500 Å to 4,900 Å. Check plants showed no response when irradiated for the same period as the test plants.

Only a few grafts, of the many tried, were successful, but when they were successful, union took place in two or three days. Neither plant was irradiated after the graft was made. The graft union was made between hypocotyls in some cases and between epicotyls in others. Both plants developed normally according to the previous treatment; that is, they reacted exactly like plants that had been similarly treated but not grafted. There was no evidence to show that any material formed by the irradiation of the one plant and capable of causing growth changes crossed over the graft union and affected the plant that had not been irradiated. On the other hand nothing from the non-irradiated plant seemed to affect the growth of the irradiated plant. The grafting operation itself did not seem to stunt or alter growth.

Avena curvature assays of hypocotyls of irradiated and non-irradiated bean plants

Emerging kidney bean seedlings were irradiated at 2,500 ergs/cm.^{2}/sec. for 15 minutes daily for five days. The hypocotyls of the irradiated plants and those of similar plants that had not been irradiated were still elongating at this time. Eleven hypocotyls from the irradiated plants averaged 19 cm. in length and weighed 30.8 grams. Six hypocotyls from the non-irradiated plants averaged 28 cm. in length and weighed 29.8 grams.

Approximately 30 grams of fresh hypocotyls cut in half lengthwise were immediately placed in 1,500 ml. of freshly prepared peroxide free ether and

allowed to stand for 22 hours at 1° C. The ether was evaporated off in a steam bath and the extract taken up in a special agar preparation and diluted so that one ml. of the agar contained the extract of 10 grams of hypocotyls. Further dilutions were also made. The extract taken up in the agar was then tested for auxin activity on Avena coleoptiles in co-operation with LINK and EGGERS (**4, 10, 12**) and using their technique and equipment. Checks using several concentrations of pure synthetic indole acetic acid were also run at the same time. The degree of curvature with the indole acetic acid was linear with concentrations up to 40 γ per liter.

Two separate series were run with very similar results and the average of these is given in table XII. The data are presented both on the basis of

TABLE XII

AUXIN CONTENT OF HYPOCOTYLS

TREATMENT	NO. HYPOCOTYLS	AV. FRESH WEIGHT HYPOCOTYL	AV. LENGTH HYPOCOTYL	AVENA CURVATURE 10 GM. HYPOCOTYL/GM. AGAR	AVENA CURVATURE ONE HYPOCOTYL/GM. AGAR
		gm.	*cm.*	*degrees*	*degrees*
Non-irradiated	12	5.1	27	6.8	3.5
2500 H 15 min.	21	2.8	20	14.7	4.1

the amount of Avena curvature when the extract of 10 grams of fresh hypocotyls was taken up in one ml. of agar and also on the basis of the amount of curvature that would result if one average hypocotyl were extracted and the entire extract taken up in one ml. of agar.

On a fresh weight or length basis the hypocotyls of the irradiated plants contained much more ether extractable auxin than did the hypocotyls of the non-irradiated plants. When the results were calculated on the basis of the amount of ether extractable auxin per hypocotyl the differences were very small, but the hypocotyls of the irradiated plants still had slightly more auxin.

Discussion and conclusions

An increase in the quantity of radiant energy was always accompanied by an increased morphological response of the plants. The increase in amount of response with increase in quantity of energy was not linear, however, but gradually decreased and became smaller as the quantity of energy became larger and larger. It has been suggested that the response increases as the logarithm of the quantity of incident radiant energy. Such a simple relationship was not found.

The results given in tables III and IV show that high irradiances were more effective than low irradiances even though the total quantity of radiant energy was kept constant. These results are in contradiction to TRUMPF'S

findings, but are in agreement with WENT's recent results. This departure from BUNSEN and ROSCOE's Law may be in part due to the masking effect of chlorophyll. Long exposures to low irradiances caused more chlorophyll to be formed than short exposures to high irradiances. Since chlorophyll absorbs radiant energy strongly in the region of the spectrum used it could act as a filter and decrease the effective irradiance beneath the plant surface. Table VII shows that measurable quantities of chlorophyll were formed under exposures as short as 15 minutes daily. Numerous observations showed that bean seedlings exposed for longer than 15 minutes daily greened much more than those given only 15 minutes daily. The latter appeared yellow in color, but the former were varying shades of yellowish green and green.

The plant response was greatest when the plants were irradiated twice daily. These results are similar to those found by WENT with peas. Irradiations less frequent than twice daily caused a lessening of the response even though the total quantity of radiant energy which the plant received during the experiment remained constant. As the number of irradiations increased over twice daily the amount of chlorophyll increased rapidly. The filtering effect of the chlorophyll and the consequent lower irradiance in the tissues may account for the decreased response of many plant parts under these conditions which were favorable to chlorophyll formation.

When the plants were irradiated less than twice daily the response decreased as the frequency of irradiation decreased. There is not enough information available to give a specific explanation of this phenomenon, though several general explanations may be found in the field of photochemistry.

The data show that the Q_{10} of the "light reaction" is as close to unity as the accuracy of this type of experiment would enable one to measure. If there were several photochemical reactions following one another rapidly in succession with chemical reactions in between, then during irradiation at high irradiances this entire chain of reactions would be slowed down to the rate of the slowest chemical reaction, especially at low temperatures since the rate of the chemical reactions would be decreased to one half or one third for every 10° C. temperature drop. The data indicate that the condition described above does not obtain and suggest that only one photochemical reaction is involved.

Previous published work indicated that only that part of the plant which is irradiated responds to the radiant energy. No clear evidence of complete localization of the response to radiant energy was found, though each part of the plant which was irradiated was capable of responding to radiant energy. The leaves near the tip and especially the growing point were the regions where the greatest overall response was obtained when they alone were irradiated. When the hypocotyl alone was irradiated, the response of any part of the plant except the hypocotyl was very small.

The grafting experiments gave no evidence that there was a substance formed in an irradiated bean seedling which would diffuse across a graft union in sufficient amounts to affect the growth of a non-irradiated bean seedling grafted to it or *vice versa*.

Albino maize seedlings which appeared to be free of chlorophyll and the carotenoid pigments responded to radiant energy very similarly to the normal seedlings of the same parentage. No extractions were made from the albino plants but it is probable that minute traces of pigments were present which could absorb the longer wavelengths of the visible spectrum.

The Avena curvature assay experiments were carried out specifically to find out whether a material causing Avena curvatures could be extracted and correlated with the quantity of radiant energy or the elongation of the internodes of kidney bean seedlings. In this study fresh, longitudinally split hypocotyls were extracted with cold, freshly prepared, peroxide free ether. There was evidence that some Avena curvature inhibitors were being extracted as well as auxin, since dilutions of one-tenth and one-fiftieth of that given in table XII gave curvatures greater than they would have been if the curvature response were as proportional to concentration as it was to indole acetic acid.

Ether extracts from kidney bean hypocotyls that were still elongating showed that variations in elongation of kidney bean hypocotyls due to irradiation with energy in the longer wavelengths of the visible spectrum are either negatively correlated with ether extractable auxin concentration as measured by Avena curvature or are non-correlated. The evidence obtained in this experiment does not indicate that auxin concentration alone can be used to explain the lessened elongation of hypocotyls of irradiated bean seedlings as compared to non-irradiated seedlings.

Summary

1. The amount of the morphological response of kidney bean seedlings increases at a decreasing rate with increases in incident radiant energy, but the amount of the response varies depending on the manner in which the energy is increased.

2. High irradiances cause more response than low irradiances even when the total radiant energy incident on the plants is the same.

3. The maximum morphological response is obtained when the plants are irradiated two or three times daily. Chlorophyll formation increases with increases in the number of cycles of irradiation up to 144 times daily.

4. Albino maize seedlings give the same morphological response to irradiation as do normal green maize seedlings.

5. The Q_{10} of the "light reaction" seems to be unity. The temperature at the time of irradiation does not in itself affect the magnitude of the response.

6. All parts of the plant can act to some extent as perceptors of radiant energy. Adjacent non-irradiated parts respond to some degree when any one part is irradiated. The leaves and especially the growing point are the most sensitive perceptors of radiant energy.

7. No effect is observed when an irradiated bean seedling is grafted to a non-irradiated seedling if both are not irradiated thereafter.

8. Ether extracts of hypocotyls of irradiated and non-irradiated bean seedlings indicate that both have about the same amount of auxin per hypocotyl, but the irradiated hypocotyls are smaller and the concentration of auxin in them is higher.

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THE CULTURE OF ALBINO MAIZE

H. A. Spoehr

(WITH TWO FIGURES)

Introduction

A number of attempts have been made to grow albino plants by means of artificial nutrition, but so far as I am aware, in none of these have plants been grown to maturity or with any material gain in dry weight. Knudson and Lindstrom (**6**) succeeded in keeping albino maize plants alive when supplied with sugar, and noted the formation of five to seven leaves, while the check plants had only two to three leaves. But the albino plants failed to make a sustained growth or to show a marked increase in weight. By the use of a sterile culture method Rischkow and Bulanowa (**12**) were able to maintain albino seedlings of *Artemisia vulgaris* alive for three months with the formation of three to four leaves. The seedlings were grown in test tubes containing mineral nutrient and glucose.

In the attempts just cited the experiments were so arranged that dependence was placed upon the capacity of the roots to absorb sufficient sugar for the development of the plants. However, this method in principle offers little promise of success, for it is known from earlier experiments that organic matter supplied to the roots cannot compensate for the lack of photosynthetic activity of the leaves of higher plants [Cerbian de Besteiro and Michel-Durand (**1**)]. The roots of plants appear to be ill adapted for the absorption of carbohydrates. At least for the practical purpose of culturing larger quantities of plants the method of organic nutrition through the roots involves an inordinate amount of labor if the culture solutions are to be kept free of infection.

That albino leaves show some characteristic differences as compared with green leaves has been shown by the chemical analyses of Church (**2**) and of Grandsire (**4**). These analyses were made primarily on variegated leaves and albino shoots. Albino leaves seem to be characterized by a relatively high water content and high ash. The ash contains a remarkably high percentage of potassium and a striking deficiency of calcium; there appear to be no significant differences in iron. The older analyses by Church of nitrogen compounds may be open to some doubt, but both Grandsire (**4**) and Schumacher (**14**) reported albinos as showing a relatively higher content of soluble nitrogen compounds and a deficiency of protein nitrogen. Similarly, albinos are low in organic matter, in carbohydrates and in organic acids. Schumacher (**14**) concluded that the relative deficiency of protein was a secondary effect, because by feeding carbohydrates to these leaves a

decided increase in protein content was observed. It is rather remarkable that the dry albino leaves yield more material extractable with absolute ether than the corresponding green leaves. Little is known, however, regarding the nature of the organic material in albinos.

Methods of culture

The investigations here reported were carried out with albino maize seedlings.[1] Only the pure white seedlings were used in both water and sand cultures. Of the hundreds of seedlings which were grown in the course of three years under a variety of conditions of light, temperature, and mineral nutrients, no tendency of the pure white seedlings to form chlorophyll was ever observed. There was, however, a considerable variability in the vigor of individual albino plants. This manifested itself in that some plants could be kept alive through artificial nutrition for 3 to 4 months, and during this time produced both types of inflorescence, whereas other plants, raised from the same lot of seed and under identical conditions, languished and died after a few weeks or produced only one inflorescence. Such variation among individuals in inbred strains is well known to geneticists. In order to bring some plants to maturity it was therefore necessary to start with a considerable number of seedlings.

For the water culture experiments the seeds were first sterilized. Several methods were tried: calcium hypochlorite, chlorine water, ultraviolet light, and "Semesan." The seeds were sprouted on sterilized filter paper. When it became apparent which seedlings were albino they were transferred to 0.5- or 1-liter wide-mouth Erlenmeyer flasks provided with rubber stoppers. The seedlings were packed by means of cotton in a hole of the stopper with the roots immersed in mineral nutrient solution. Although a number of seedlings started in this manner finally grew to maturity, in general the water culture method did not prove to be entirely satisfactory because, in spite of various methods of sterilizing the seeds, a large number of infections developed in the endosperms and often spread to the root system.

More satisfactory results were obtained with sand cultures which for the present purposes were much simpler to start and to maintain. The seeds, without being sterilized, were sprouted in clean sand, and as soon as it was apparent which were albino seedlings these were transplanted in sand in 4- or 6-inch pots. The pots were placed on glazed saucers and the mineral nutrient solution (or water) was added to these saucers so that the top of the sand in the pots was always dry. These cultures showed the best growth and there were no evidences of disturbing effects due to infection.

[1] The original lot of seed for these experiments was obtained through the kindness of Maize Genetics Coöperation of Cornell University, for which I wish to express my sincere appreciation.

Stock solutions of mineral nutrients were prepared as follows, each separately in 1000 ml. of distilled water: 5 gm. KCl; 5 gm. KNO_3; 28.75 gm. $Ca(NO_3)_2 \cdot 4H_2O$; 10.25 gm. $MgSO_4 \cdot 7H_2O$; 2.5 gm. K_2HPO_4 plus 2.5 gm. KH_2PO_4 adjusted to a pH of 6.6 to 6.7. Of these five stock solutions 200 ml. of each were added to four liters of distilled water and the following additions were made to make up the mineral nutrient solutions used: 10 ml. of a solution of 16.65 gm. $FeCl_3 \cdot 6H_2O$ in 1 liter of water; 1 ml. of a solution of 0.157 gm. $MnCl_2 \cdot 4H_2O$ in 1 liter of water; and 0.1 ml. of a solution containing 2.86 gm. H_3BO_3, 1.81 gm. $MnCl_2 \cdot 4H_2O$, 0.22 gm. $ZnSO_4 \cdot 7H_2O$, 0.08 gm. $CuSO_4 \cdot 5H_2O$, 0.072 gm. MoO_3 in 1 liter of water. In the nutrient solution prepared in this manner green seedlings made good growth in the light in water cultures as well as in sand cultures.

A second mineral nutrient solution was tried in which the potassium phosphate mixture in the solution just described was replaced with an ammonium phosphate mixture of pH 6.7. It was thought that the ammonium mixture might be more favorable for protein synthesis and consequently for the general development of albino plants. This, however, was found not to be the case.

After many efforts to achieve satisfactory growth of the albino seedlings by the method of supplying them with sugar added to the mineral nutrient solution, this method was abandoned as unsuitable. Recourse was taken to what would appear to be a more natural method, that of supplying the plants with organic nutrients through the leaves. The tip of the leaf was cut off and the portion attached to the plant was immersed in a sucrose solution contained in a glass vial, usually 1 cm. in diameter; this was then stoppered with a wad of cotton. See figure 1. The vials were held in coils of wire attached to supports which could be adjusted in every direction. Various sugars and mixtures of different sugars were tried. The experiments here described were carried out entirely with sucrose, which yielded the best results. By empirical methods a concentration of 0.3 molar sucrose was determined as giving the best growth effects. The method is of course, not a strictly sterile one, but precautions were taken to use sterilized scissors, and the vials, after being carefully washed, were kept in an oven at 100° C., and the sucrose solution was sterilized before use. Every other day, or every day during warm weather, the sucrose solutions were replaced by removing the vial, cutting off about 0.5 cm. from the end of the leaf and immersing it in a fresh solution of sucrose in a clean vial.

In an effort to attain as rapid and as great a growth as possible of the albinos, it was thought that this might be aided by the addition of some of the accessory factors which have been found to stimulate growth. Experiments were carried out in which some of the following, singly or combined, were added to the sucrose solution: ascorbic acid, vitamin B_1, vitamin B_6,

adenine, and indolylacetic acid. No significant differences were noted in plants thus fed as compared with the controls which were fed sucrose only.

FIG. 1. Albino maize plant, in sand culture, grown with 0.3 molar sucrose solution as only organic nutrient, in 4-inch pot.

It is probably important in this connection to give consideration to the great variability in the albino stock itself, so that it could probably be expected that significant differences could be noted only in experiments with large

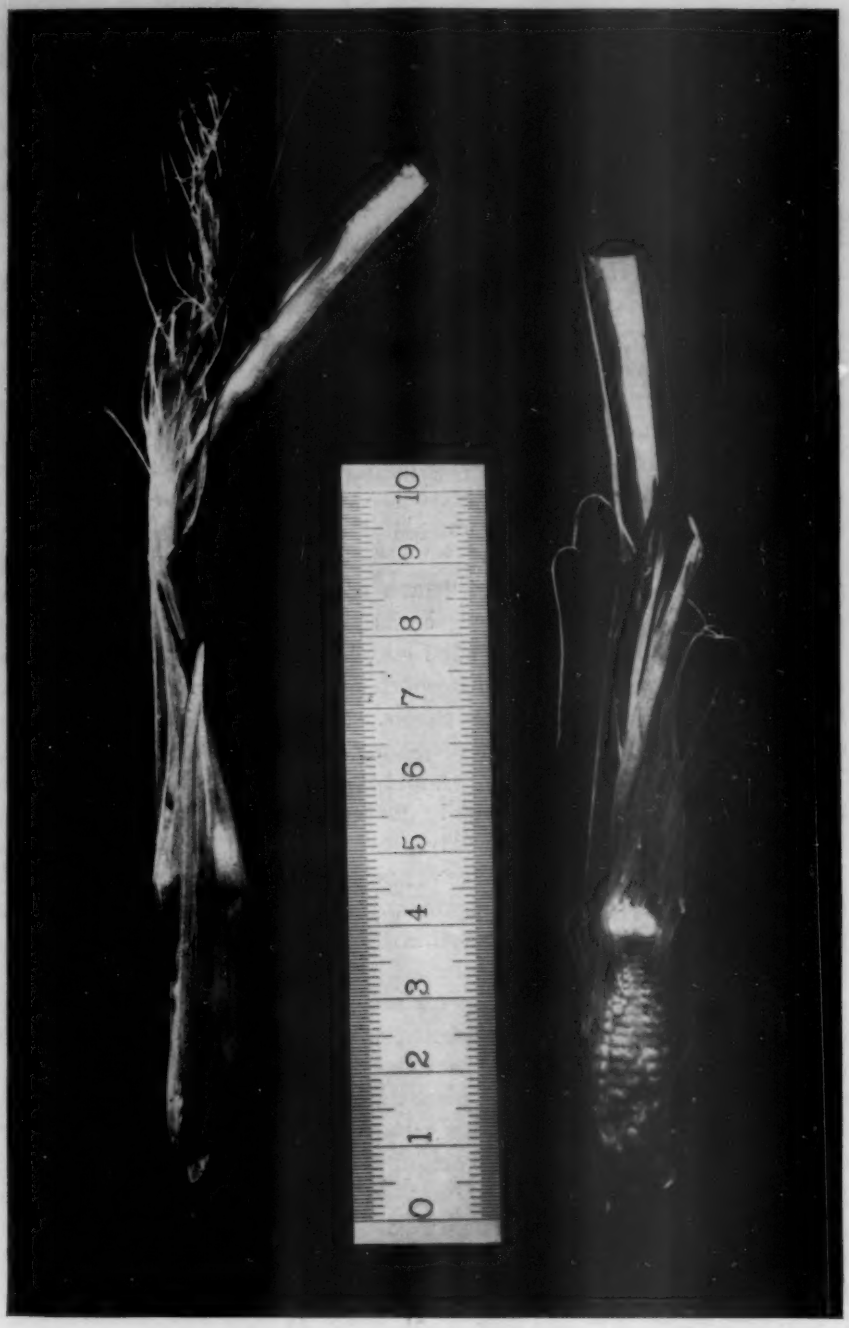

FIG. 2. Ears of albino maize plant grown with 0.3 molar sucrose as only organic nutrient, 10-cm. scale.

numbers of plants. It is also possible that there were other, unrecognized factors which were more limiting for the growth of the plants than the growth-promoting materials which were added to the sucrose solution.

In the endeavor to determine the most favorable conditions for the culture of albino plants through artificial organic nutrition, it was found that the form and rate of growth of these plants are strikingly affected by light. Albino plants were grown under three sets of light conditions: in the greenhouse, in diffuse north light, and in the dark. The temperature in all of these ranged from 18 to 25° C. Of necessity the dark series was exposed to some light, because about one-half hour of illumination with a 25-watt lamp was required every day to replace the organic nutrient solutions. This was not sufficient to maintain or produce chlorophyll in normal etiolated maize plants. The plants grown in the greenhouse in the spring and summer months were quite dwarfed and attained a height of not over six inches. They produced 10 to 15 leaves and none of these was over six inches in length. The addition of indolylacetic acid to the organic nutrient solution did not improve the growth of these plants.

The albinos grown in the dark exhibited the elongation typical of etiolation, with long, slender, delicate leaves and narrow, weak stems. These plants were kept alive over four months and produced rudimentary ears. They attained a height of 80 to 100 cm., but because of their long, weak internodes they were difficult to support. Here also the addition of indolylacetic acid produced no visible effects. Alongside of these albinos some chlorophyllous seedlings were also grown by artificial organic nutrition. The latter soon lost their chlorophyll and the new leaves were free of chlorophyll, so that these plants could not be distinguished from the albinos. After having been in the dark for 125 days, during which period they produced 10 to 14 leaves, all the plants were exposed to the light of 250-watt Mazda lamps for 12 hours. The plants which originally had been green, formed perceptible amounts of chlorophyll within two hours, while the albinos showed no change in color.

The objective of these experiments was to determine the most favorable conditions for culturing albino plants, which could then be subjected to chemical analysis. For this purpose the best results were obtained under conditions of diffuse light. The plants grown under these conditions were sturdier, had broader leaves with a strong midrib, and more of the plants survived. They grew to 40 to 70 cm. in height in 120 days. Some of them produced both pistillate and staminate inflorescences, the latter with considerable pollen; other plants produced only pistillate inflorescences and from these the silk grew 10 to 20 cm. long. Finally, some plants produced no inflorescences at all, and about one-half of the seedlings for no apparent reason died before they had reached an age of 90 days.

Results

The experiments have demonstrated that it is posible to maintain cultures of albino maize plants by means of artificial organic nutrition and that under favorable conditions the dry weight of these plants is considerably higher than that of the seeds. Beyond this fact the data probably have little quantitative significance.

The gain in dry weight

To illustrate the magnitude of this gain in organic matter by the artificially nourished albino plants the following experimental results are presented. One hundred maize seeds, dried *in vacuo* over calcium chloride, showed a mean weight for each seed of 0.20003 grams with a standard deviation of 0.04097 grams and a standard error of 0.004097 grams. While the calculations of the percentage gain in dry weight of the nourished plants over the seeds (table I) is based upon this mean weight, it must be remembered that the artificial nutrition of the plant does not start from this stage. During the germination of the seeds and the growth of the seedlings to a stage where they can be artificially nourished through the leaves, material contained in the endosperm is being consumed. Thus, thirteen sturdy albino seedlings, 12 days after sowing, were dried at 97° C. for 30 minutes and then *in vacuo* over calcium chloride. They had a mean dry weight of 0.15904 grams, representing a loss of the original dry weight of the seeds of about

TABLE I

Gain in dry weight of artificially nourished albino maize plants calculated on the basis of the mean dry weight of the seed. Plants grown in sand culture at 22 to 26° C. in diffuse light

Growing period	Days	Fresh weight	Dry weight	Percentage dry weight	Calculated percentage gain in dry weight
		gm.	*gm.*	%	%
May 29 to Sept. 29	123	8.80	1.10	12.5	450
" " " " "	123	4.70	0.72	15.3	265
" " " " "	123	3.51	0.70	19.9	250
May 29 to Oct. 3	127	12.00	1.90	15.8	855
" " " " "	127	4.50	0.50	11.1	150
" " " " "	127	12.00	1.53	12.7	665
" " " " "	127	17.01	1.62	9.5	710
" " " " "	127	12.20	1.54	12.6	670
May 29 to Oct. 16	140	.....	2.92	.....	1365
" " " " "	140	.....	0.56	.....	180
" " " " "	140	.....	1.20	.....	500
" " " " "	140	.....	1.89	.....	845
" " " " "	140	.....	1.39	.....	595
" " " " "	140	.....	1.30	.....	550

20 per cent. The standard deviation in the dry weights of the albino seedlings was 0.05337 grams and the standard error 0.014802 grams.

By the method of culture of the albino plants here followed they were subject to further material losses which are also not included in the recorded percentage gain in dry weight. Each day a few millimeters to a centimeter was cut from the end of the leaves submerged in the nutrient sugar solution, so that eventually, especially in the older leaves, there was a loss of about 50 per cent. of the leaf weight. This is estimated to amount to about 10 per cent. of the entire weight of the plant. Consequently the calculated percentage gain in dry weight of the artificially nourished plants represents an underestimate of the total gain.

As has already been mentioned the albino seedlings show a considerable variation in vigor and this is reflected in the great range of the percentage gain in dry weight, varying from 150 to 1365 per cent. in the example cited. Needless to say these gains in dry weight are very much smaller than are those made by a normal plant which obtains its organic nutrition by means of photosynthesis. There are doubtless many improvements in the methods of culture which are still to be worked out. The factor of temperature alone may be cited as deserving of special study. In one series of cultures the temperature range was between 12° and 20° C. for 102 days and the plants appeared to be in good condition. But, although they had grown and put out new leaves, so far as the dry weight was concerned the plants just about held their own, because in all this time there was no appreciable gain in dry weight over that of the seeds.

Starch formation in albino leaves

Relatively little study has been given the organic nutrition of albino or chlorotic leaves. It has been known for a long time that, although the chromatophores of albino, chlorotic, and albescent leaves do not form starch in the light, starch is deposited in these organs when such leaves are floated on solutions of sucrose or glucose, as was demonstrated by Saposchinkoff (**13**) and Zimmermann (**18**). Winkler (**17**) also demonstrated that leaves of *Fagopyrum tataricum* and of *Zea mays*, which were white through withholding iron from the nutrient solutions, did not produce any starch in the light, but formed abundant starch when placed on solutions of glucose or sucrose. In our own experiments we have repeatedly been able to observe the formation of starch in albino maize leaves when these were fed sucrose.

In this connection an observation first made by Saposchnikoff (**13**) may be of significance. Normal green leaves are able to form starch in the dark not only from sugars such as glucose, fructose, galactose and sucrose, but also from such compounds as glycerine and mannitol which represent a slightly higher level of reduction than the sugars. Saposchnikoff found that the

chlorophyll-free portions of variegated leaves do not form starch from glycerine and mannitol. We have also found that albino maize leaves do not form starch from glycerine or sorbitol. In table II the results are summarized of comparative experiments with green and albino maize leaves. The leaves were kept in the dark until they were free of starch. A number of leaves were then submerged in the solutions of sugars, glycerine, and sorbitol, and placed in a vacuum desiccator. By careful evacuation the air was removed from the leaves which thereby became infiltrated with the respective solutions of organic nutrients. This process required about 15 minutes. Air was admitted into the desiccator and the leaves removed to vials containing enough of each of the solutions so that the cut ends of the leaves were

TABLE II

STARCH FORMATION BY GREEN AND ALBINO LEAVES OF *Zea mays* INFILTRATED WITH SOLUTIONS OF VARIOUS ORGANIC NUTRIENTS IN THE DARK

Solutions	*Zea mays*	
	Green	Albinos
0.3 M. sucrose	+	+
0.1 M. sucrose	+	+
0.3 M. d-glucose	+	+
0.1 M. d-glucose	+	+
0.3 M. glycerine	+	−
0.1 M. glycerine	+	−
0.3 M. d-sorbitol	+	−

well submerged. The leaves were kept in the dark at 22 to 24° C. and tested for starch after 24 and 48 hours.

This difference between albino and chlorophyllous leaves may be of more fundamental importance than has been realized. Starch formation from compounds such as glycerine and sorbitol indicates that normal leaves have the capacity to oxidize an alcohol group, probably forming a keto- or aldosugar. Albino leaves apparently lack this capacity. The formation of chlorophyll is also associated with oxidative reactions. Whether there is any connection between the lack of chlorophyll in albinos and their apparent incapacity to oxidize compounds such as glycerine remains to be established.

In agreement with the results of GRONER (**5**) it was found that there is very little difference, if any, between the rates of respiration of albino and chlorophyllous leaves. In these experiments seedlings were used which drew upon the endosperm for their supply of carbohydrate. On the other hand, SMIRNOW (**15**) and SCHUMACHER (**14**) found that the white portions of variegated leaves had a lower rate of respiration than the portions containing chlorophyll, and SCHUMACHER was able to demonstrate that this lower rate was due to a deficient supply of carbohydrate. Beyond this, however,

it appears to be quite well established from the investigations of SMIRNOW (**15**) and of SCHUMACHER (**14**) that the white and chlorophyllous portions of leaves differ in regard to the activity of certain enzymes which are presumed to be associated with oxidative processes of the cells. The chlorophyll-free tissue contains more peroxide and less catalase, and SCHUMACHER concludes that this is an indication of a lower or more sluggish rate of energy transfer. Until the rôle of these enzymes in the oxidative metabolism has been more clearly established there seems little prospect, however, of drawing definite conclusions from such data concerning the differences between the two types of tissue.

LEBEDEFF (**10**) made comparative studies of various functions of green and albino maize plants, including rate of phototropic response, rate of growth, swelling of the seeds, rate of respiration, and metabolism of nitrogen compounds. He concluded that the two forms of leaves did not differ in their assimilating mechanism but only in their capacity to utilize radiant energy.

Discussion

It has been generally assumed that in the photosynthetic process the carbon dioxide is reduced to the level of a carbohydrate. In any consideration of the chemical mechanism of this process it is obviously of importance to know whether carbohydrates are the only products formed, or whether simultaneously other products, such as hydroxyacids, fatty acids and hydrocarbons are also produced directly through photosynthesis. The concept that the carbon dioxide is reduced to the level of a carbohydrate rests essentially upon the results of chemical analysis and of measurements of the photosynthetic quotient.

It must be admitted that the enormous effort which has gone into determining the nature of the photosynthetic products of higher plants by means of chemical analysis, extending over fifty years, has not been very rewarding. No reliable information has been gained by this means as to intermediate products nor regarding the question whether there is a specific carbohydrate formed in the process. It is doubtful whether a solution of these problems can be gained without recourse to special means such as are afforded by the use of radioactive isotopes of carbon. It is primarily the question as to what percentage of the carbon dioxide absorbed in photosynthesis appears as carbohydrate. A definite answer to this question is still outstanding (**9**).

The observation that the photosynthetic quotient is very close to unity has been commonly assumed to mean that the carbon dioxide has been reduced to a carbohydrate (**16**). This interpretation is, however, open to some question. In the first place, a value of unity has usually been obtained only after the plant has been subjected to illumination for some time. During the first minutes of illumination the quotient is irregular and shows con-

siderable deviation from unity (**5, 7, 11**). No entirely satisfactory explanation has as yet been offered for these fluctuations. Whether, for example, these deviations from unity in the value of the quotient signify the formation of intermediate products, which are subsequently further reduced, or whether it is an indication that during this period other products are predominantly formed, with a quotient higher or lower than unity, remains uncertain. Furthermore, the fact must be borne in mind, that in the measurement of the photosynthetic quotient the gas exchange of an over-all reaction is being determined. From these measurements alone there is nothing which precludes the concept that the value of the quotient represents the net result of the gas exchange of a number of reactions. Whether these reactions all proceed to the same end product is not known. But there is no evidence which precludes the possibility that the photosynthetic process consists of serial reactions, proceeding simultaneously and resulting in products of different nature, *i.e.*, of a different level of reduction. Each product would have a different individual photosynthetic quotient, and still the total or over-all quotient could be unity. For example, if in the photosynthetic process a mixture were formed consisting of a hydrocarbon with a quotient of less than one, a carbohydrate with a quotient of one, and a hydroxyacid with a quotient greater than one, the total gross reaction might still yield a photosynthetic quotient of unity.

If it were established that the photosynthetic process resulted directly in the synthesis of a variety of products of varying level of reduction instead of only carbohydrate, it would materially affect many theoretical concepts of the mechanism of the process. If carbohydrate were the only substance formed in the photosynthetic process, the commonly accepted opinion would hold, namely, that the great array of compounds found in plants arises from carbohydrate through biosynthetic reactions. On the other hand, if carbohydrate is not the only product of photosynthesis, even though the substances of higher and lower level of reduction are formed in relatively small amounts, our concepts of the mechanism of the photosynthetic reactions and of the organic nutrition of plants would have to be materially modified. Finally, it is conceivable that the photosynthetic and chemosynthetic reactions occurring in the plant are so inextricably intermeshed that the unravelling of this tangle and the elucidation of any one of these reactions may prove to be a far more complex problem than we now realize.

The theory that carbohydrate is the only product of photosynthesis and that the entire organic nutrition of the plant can ultimately be satisfied from this material can, in a measure at least, be subjected to experimental test. It has long been known that some of the lower plants, particularly the algae, can be kept alive for extended periods of time in the dark when cultured, in solutions of carbohydrates and other organic compounds. Similarly, the

leaves of higher plants are known to form starch from a variety of carbohydrates. It appears that the higher plants are considerably more selective in respect to their artificial organic nutrition than are algae (**8**). By entirely excluding photosynthesis a test could be made to determine to what extent a higher plant can be brought to full development by means of artificial nutrition exclusively with a carbohydrate. Obviously two means are available for such an experiment: the culturing through artificial nutrition of normal, chlorophyllous plants in the dark, and the culturing by the same means of albino plants. Experience has demonstrated that the use of normal plants in the dark was considerably complicated by the "etiolation" effects produced by culturing the plants in the dark. On the other hand, albino plants can be grown in the light and all "etiolation" effects can be thus avoided; at the same time these plants are devoid of the capacity for photosynthesis.

It is, of course, difficult to establish criteria on which an unequivocal answer of this test can be based. As a beginning the simplest and most rational criterion has been the gross development of the plant, including the length of time it is kept alive, the number of leaves produced and the development of sex organs. This could then be supplemented with chemical analysis of the constituents of the artificially nourished plants as compared with normal ones. But for this latter purpose a sufficient amount of plant material must be available. Consequently, the chief effort for the present has been to determine the most favorable conditions for the culturing of albino plants by means of artificial nutrition.

The contrasting properties and the differences in their capacity to utilize compounds such as glycerine and sorbitol, exhibited by albino and chlorophyllous plants, emphasize the necessity of exercising caution in drawing conclusions regarding organic nutrition on the basis of the method which is here being considered. Associated with their lack of ability to form chlorophyll, other deficiencies may exist in albino plants which might make it impossible for them to carry on certain reactions occurring in chlorophyllous plants. It is conceivable, for example, that albinos lack not only the capacity to form chlorophyll, but that linked with this deficiency, are also other deficiencies affecting their metabolism.

Summary

It has been demonstrated that albino maize plants can be kept alive for several months by artificial organic nutrition with sucrose. These plants showed a definite increase in dry weight above that of the seeds from which they sprang. They produced the same number of leaves as normal green plants and also staminate and pistillate inflorescences. The albino leaves formed starch in the dark when infiltrated with solutions of sucrose and dex-

trose, but, in contradistinction to green leaves, not with solutions of glycerine or sorbitol. Some theoretical aspects of the use of cultures of albinos for elucidating certain phases of the photosynthetic problem are discussed.

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ABSORPTION AND ACCUMULATION OF RUBIDIUM BROMIDE BY BARLEY PLANTS. LOCALIZATION IN THE ROOT OF CATION ACCUMULATION AND OF TRANSFER TO THE SHOOT

F. C. Steward, P. Prevot, and J. A. Harrison[1]

(with two figures)

Although many have investigated the distribution of water absorption along the axis of roots (**5, 7, 8**), investigations on the localization of ion intake and accumulation are few and there seems to have been no attempt to determine whether all the salt content of a root system is equally accessible to the shoot. There is still the tendency to regard any unsuberized root surface bearing root hairs as a potential absorbing surface (**2**); and to regard the problems of penetration of the surface, rather than of intake by cells and transfer to the shoot, as the major ones.

Prevot and Steward (**6**) investigated the uptake of bromide ion in bromide solutions by unbranched roots of barley (*Hordeum vulgare*) with special reference to the distribution of the absorbed salt along the axis of the root and the effect upon this distribution of time and the nutritional conditions during growth.

In an unbranched root of barley there exists a steep gradient of activity along the axis. Near the apex are meristematic and elongating cells, still actively growing, which attain the highest concentrations in the root; at distances up to several centimeters (until branch roots appear) the mature cells have declining activity in growth and salt accumulation. The graded activity in salt accumulation, recognized by Prevot and Steward, was regarded as an inevitable consequence of the progressive development of cells from the root apex, where they are in rapid growth and active metabolism, to maturity when they are fully expanded and, in a monocotyledon, no longer able to grow.

Modern knowledge recognizes that the distribution of growth in roots may be associated with, even if it is not explained by, the unequal content of growth substances which are more concentrated near the root apex (**1, 12**). Whatever the ultimate cause of the declining growth rate proximally from the root apex toward its base, the evidence of concomitant decrease in salt accumulation is consistent with the general picture of the process of salt accumulation which has emerged from work on Nitella, on storage tissues,

[1] This paper was prepared by the senior author. At his request the undersigned attempted to transmit a copy of the manuscript to Dr. Prevot for his criticism. Because of war conditions, no communication has been possible. It is therefore deemed desirable to submit the article for publication without further delay.—D. R. Hoagland.

and on root systems. ROSENE claims that the base of the root absorbs more water than near the apex (7).

In 1939, GREGORY and WOODFORD (3) described an apparatus, applicable to the primary root of *Vicia faba*, for the comparison in respiration and absorption of successive segments along the axis of this rather massive root. PREVOT and STEWARD showed that, when isolated from the shoot, the primary root of *Vicia faba* could accumulate bromide (concentration in the root of the order of two to three times that of external solution which was 0.005 equivalents Br per liter) but that it was much less active in this respect than the barley roots they used, although somewhat stimulated if the shoot was previously exposed to the light. GREGORY and WOODFORD succeeded in measuring directly the oxygen intake along the length of the attached primary roots of *Vicia faba*. They showed that the apical segments had the greatest respiration rate and that the rate decreased rapidly from the apex backwards. They thus produced direct quantitative evidence of a gradation of respiratory activity in *Vicia faba* along the axis of the root which in the work of PREVOT and STEWARD was only detected qualitatively by the use of indicators of oxidation and reduction. Since then Prevot[2] has applied manometric methods and also showed that the respiration of isolated segments of barley root was greatest the nearer these were to the root apex. GREGORY and WOODFORD found that the attached primary root of *Vicia faba*, like the detached ones used by PREVOT and STEWARD, were somewhat sluggish in absorption and, although the apical segment absorbed more nitrate than the older ones, the fate of the absorbed nitrate after absorption, *i.e.*, whether retained in the cells of the segment or transferred to the shoot, was not investigated and accumulation of the absorbed solute did not occur in the tissues during the experiment.

PREVOT and STEWARD made their observations only with reference to the bromide ion and, to avoid the complications of the removal to the shoot, on excised roots. Since then STEWARD and HARRISON (1) have perfected methods for the analysis of rubidium and have investigated the uptake of rubidium bromide by potato discs. It was shown that the intake of the cation (Rb) is affected by similar variables to that of the anion (Br) and could, therefore, be used as a cation indicator of salt accumulation with many of the advantages possessed by the anion bromide. Such differences as were observed in the uptake of rubidium and bromide were attributed to a brief intake of rubidium, unaccompanied by anion, due to a mechanism (perhaps peculiar to potato discs) in which vital activity was not concerned.

In the present investigation free use was made of the methods of STEWARD and HARRISON and the opportunity was taken to investigate the distribution

[2] Results communicated to Congress de Societe Francaise pour l'avancement des Sciences, 1939.

of the accumulation of cations in the root (which had hitherto rested upon the qualitative observations of others) and to ascertain the region in the root from which the shoot can, most readily, withdraw salt.

Normally, during growth in contact with a plentiful supply of nutrients, the cells far from the root apex retain much stored salt. When grown with a severely restricted supply of salt, roots may be obtained which, due to their initial deficit, can absorb salt (bromide) to high concentrations even far from the apex (**4**). The question, therefore, arises whether the apical regions, which most readily accumulate the salt *de novo*, also retain it most tenaciously in competition with the shoot. The problem has anatomical implications since the cortex in the older parts of monocotyledon roots, in which the bulk of the absorbed salt is stored, may be separated from the stele by an endodermis which is suberized and with few, or no, passage cells. This reason alone might—as PRIESTLEY and SCOTT hypothesize (**10**)—preclude the older parenchyma of the root from transferring salt to the shoot. An attempt was made to throw some light on this problem by quantitative tests.

Method and results

THE GRADATION OF CATION ACCUMULATION IN BARLEY ROOTS

This was investigated by methods similar to those used by PREVOT and STEWARD using grain from the same stock. The fresh weight of the samples of the root segments (30 or 50 to a batch) was recorded, the salts were thoroughly extracted in water, the extracts evaporated, and then redissolved in a volume of water which represented a definite multiple ($\times 5$ or $\times 10$) of the total water in this sample. Aliquot parts (0.02 or 0.05 ml.) of this solution were then treated as described by STEWARD and HARRISON for the spectrographic determination of rubidium.

Data were obtained after absorption by excised roots during 25 and 50 hours from 0.005 M rubidium bromide. These data (table I) are illustrated in figure 1 in which the concentration observed in each segment is plotted as at the midpoint.

The similarity in the data for rubidium absorption in table I and figure 1, with the earlier data for bromide uptake is evident. Conclusions concerning the relationship of growth and salt intake arrived at by PREVOT and STEWARD by reference only to an anion (Br) clearly apply also to a cation (Rb).

In the behavior of excised roots, time is an important variable for, lacking the continued stimulus of the shoot, they eventually pass into decline after periods which vary with their previous nutritional treatment. It should be noted that in the data of table I, the differences as between the apex and the older segments become more marked after the elapse of time which is necessary for the activity of the growing region to operate.

The gradation of cation accumulation along the length of the root can be seen qualitatively for both rubidium and potassium in figure 2.

Roots from the experiment of table I—after 50 hours of absorption—were cut, not into 1 cm. but into 0.5 cm. segments. Aliquot parts of the

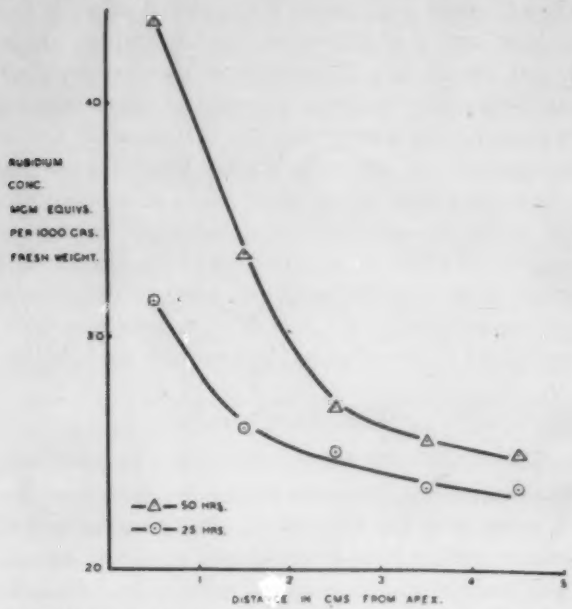

FIG. 1. Distribution of rubidium in excised roots showing the effect of time.

extracts were burned in the way prescribed for the spectrographic determination and successive spectrograms were obtained on the same plate for the ten segments into which the root was subdivided. The gradation in the intensity of the lines for potassium and rubidium was clearly evident.

TABLE I

DISTRIBUTION OF ABSORBED RUBIDIUM IN EXCISED BARLEY ROOTS—THE EFFECT OF TIME

No.	DISTANCE FROM APEX	FRESH WEIGHT*	CONCENTRATION† Rb AFTER 25 HOURS	CONCENTRATION† Rb AFTER 50 HOURS	PERCENTAGE GAIN 25 TO 50 HOURS
	cm.	*gm.*			*%*
1	0.0 – 1.0	0.0330	31.5	43.5	38.1
2	1.0 – 2.0	0.0384	26.0	33.5	28.8
3	2.0 – 3.0	0.0390	25.0	27.0	8.0
4	3.0 – 4.0	0.0384	23.5	25.5	8.5
5	4.0 – 5.0	0.0399	23.5	25.0	6.4

* From 30 roots.

† Milligram-equivalents of rubidium per kilogram fresh weight.

These determinations were made on infra red plates especially sensitive in the region concerned. From the negative plates a positive was made by printing on to Ilford Fine Line film and from this negatives were printed on to paper. The portions of the print which contained in the spectrograms the rubidium doublet (7811, 7950) and the potassium doublet (7665.3, 7699) were cut away and figure 2 reproduces these mounted alongside a sketch of the root showing the segments to which they refer. The figure shows clearly that *both* rubidium (absorbed only during the experimental period of 50 hours) and potassium (absorbed exclusively during growth) reveal the same gradation of concentration along the root. The intensities

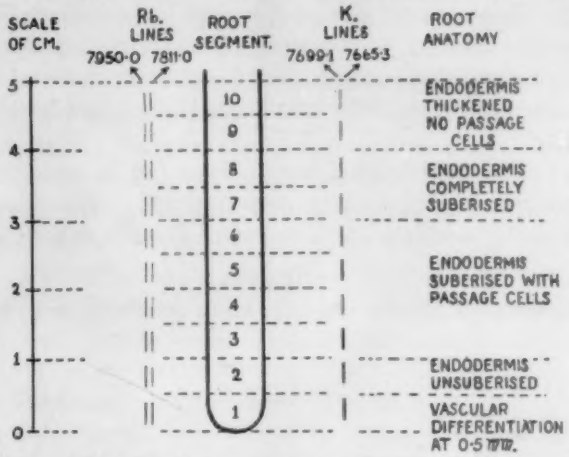

FIG. 2. The gradation of cation accumulation in roots shown spectrographically.

of all the lines increase smoothly from the lower concentrations in the older parts of the root to the youngest, 0.5 cm. near the apex (no. 1).

It was noted earlier by PREVOT and STEWARD that even a distance of 1 cm. behind the root apex includes not only the meristem, a limited zone of conspicuous cell division, but consists mainly of cells in which growth by extension predominates over division. The experiment of figure 2 is interesting because the smaller 0.5-cm. apical segments analyzed again had the greatest concentrations and, though only one stage nearer the ideal which would reveal the behavior of strictly meristematic tissue, the indication is that the nearer the segments analyzed approached the root apex the greater were the concentrations of salt which they contained. Technical difficulties in the analysis of still smaller segments are great.

ABSORPTION OF RUBIDIUM BROMIDE BY BARLEY ROOTS AND ITS REMOVAL TO THE SHOOT

Three parallel experimental series were used as follows:

SERIES I.—This consisted of barley plants grown to produce long, straight, relatively unbranched roots of low salt content, with their roots attached, and which absorbed rubidium bromide continuously from aerated 0.005 M RbBr per liter during the three periods of the experiment. In this case the absorbed salt was partitioned between the root and shoot.

SERIES II.—This consisted of plants similar to those of series I, but their shoots were excised so that the roots absorbed rubidium bromide from the external solution continuously during the experiment without the stimulus of the shoot and without transferring salt to the shoot.

SERIES III.—This consisted of plants which had their roots attached throughout the experimental period but they were transferred from the rubidium bromide solution to tap water (containing calcium) at the end of period 1. During the ensuing periods 2 and 3 any removal of salt to the shoot which occurred depleted the root of that which had been accumulated during period 1.

At the end of each period batches of roots (50 in each) were sampled for analysis in the way previously described (**6**). The concentration of absorbed ions in the segments of the roots are given in table II.

TABLE II

ABSORPTION OF RUBIDIUM BROMIDE BY BARLEY ROOTS AND ITS REMOVAL TO THE SHOOT

PERIOD NO.*	ROOT NO.	SEGMENT DISTANCE FROM APEX	SERIES I		SERIES III		SERIES II		BROMIDE CONCENTRATION IN GROWING SHOOT	
			[Rb]	[Br]	[Rb]	[Br]	[Rb]	[Br]	SERIES I	SERIES III
		cm.	†	†	†	†	†	†	†	†
	1	0.0 – 1.0	63.0	27.0	See series I		38.0	26.0		
	2	1.0 – 2.0	36.0	19.0			34.0	.....		
1	3	2.0 – 3.0	26.0	17.0			28.0	25.0	33.0	.....
	4	3.0 – 4.0	16.0	16.0			27.0	21.0		
	5	4.0 – 5.0	12.0	14.0			27.0	33.0†		
	1	0.0 – 1.0	.....	.....	32.0	3.2	29.0	35.0		
	2	1.0 – 2.0	.....	.....	19.0	1.6	24.0	28.0		
2	3	2.0 – 3.0	.....	.....	19.0	2.8	20.0	22.0	36.0	26.0
	4	3.0 – 4.0	.....	.....	14.0	3.9	17.0	24.0		
	5	4.0 – 5.0	.....	.....	11.0	6.0	18.0	30.0‡		
	1	0.0 – 1.0	77.0	32.0	22.0	0.0	35.0	32.0		
	2	1.0 – 2.0	67.0	19.0	14.0	0.0	24.0	19.0		
3	3	2.0 – 3.0	61.0	14.0	13.0	0.0	16.0	17.0	44.0	21.0
	4	3.0 – 4.0	46.0	19.0	12.0	0.0	12.0	12.0		
	5	4.0 – 5.0	39.0	18.0	10.0	0.0	11.0	14.0		

* Period no. 1 = 42 hours.
" " 2 = 48 "
" " 3 = 48 "

† Milligram-equivalents per kilogram of water.

‡ High value due to presence of secondary root initials.

Throughout table II there is an evident tendency for the concentration of rubidium, as observed in the tissue analyzed, to exceed that of bromide. Excess of cation (K) over anion (Br) absorption is a usual resultant of the technique of growing plants (Hordeum or Cucurbita) in a restricted supply of nutrient thus producing roots relatively low in salt but high in sugar. It is to be noted, however, that the apparent excess of rubidium over bromide was greatest in those cases where the shoot was attached; it thus appears that the difference was not due wholly to differential uptake of the two ions by the root system but was in part due to differences in the export to other parts of the plant. That bromide passed to the shoot more readily than rubidium is clear from the data of series III in which the decrease which occurred during periods 2 and 3 was due to removal to the shoot and is clearly more rapid in the case of bromide (note continued gain in concentration of bromide in the growing shoot among the periods 1, 2, and 3). It is not to be supposed that bromide migrated to the shoot unaccompanied by cation, for it may have been accompanied by cations (K) already in the tissues before the experiment. The essential point is that the conditions which regulate accumulation—alike in the root and the shoot—intervene and regulate, *independently* even for the two ions of an absorbed salt, the amounts of each which migrated to the shoot. This can be seen also in series I, since the concentration of rubidium in the attached roots increased with time much more than that of bromide which, despite continued absorption (see data for leaves), remained much at the level attained in period 1.

In the excised roots which lacked the stimulus of the shoot, the concentrations of rubidium and bromide did not increase progressively with time. The rubidium concentration of the attached roots, despite export to the shoot, was actually higher than in excised roots after the same period of absorption by excised roots, however, the concentration tended to fall as growth subsided and decline approached except in the apex where growth and activity lingered longest, even though the roots remained in the salt solution.

When the root was deprived of external salts (series III) and the shoot allowed to withdraw salt from the root, the total concentration in the shoot did not increase although bromide disappeared from the root; it even declined, because the quantity of bromide was insufficient to maintain the supply to the growing shoot. In terms of concentration, the export of salts, both rubidium and bromide, from the root appears greatest from the apical region; so much so, in fact, that the gradation of bromide accumulation normally found in either excised or attached roots when they are in external contact with it was actually reversed (period 2, series III) before the root became completely depleted of its normal bromide.

Ultimately all of the bromide was transferred from these roots to the

shoots and in this case there can be no question that the change in concentration truly reflects actual removal of salt. There is, however, a complication which should be mentioned. Attached roots, after contact with rubidium bromide for longer than 48 hours unexpectedly showed a marked swelling of their tips. Microscopical examination showed that this was due to expansion of cells only and not to division. In sampling, equal lengths of root were included in the different segments and the swelling effect referred to is reflected in the fresh weights of the segments cut from 50 roots. Segments which swell would, due to this, experience a fall in bromide concentration. The question arises whether the drop in concentration recorded for series III, periods 2 and 3 represents a real export of salt from the apical segments.

As the experiment was performed, it is difficult strictly to compare segments which represent the same amount of tissue in periods 1 and 2. The best approximation is obtained by regarding units of length of root tissues as really comparable and that the swelling of the apex affected only the transverse dimension of the root. On this assumption, and knowing the fresh weight of the samples, the total amount of rubidium and bromide present in the segments 1 cm. long can be calcuated (table III).

TABLE III

CALCULATION OF THE TOTAL AMOUNT OF RUBIDIUM AND BROMIDE PRESENT IN 1-CENTIMETER SEGMENTS OF BARLEY ROOTS

Root no.	Distance from apex	Fresh weight of 50	Total amount*		Total amount removed*		Conditions
			Rb	Br	Rb	Br	
	cm.	*mg.*					
1	0.0 – 1.0	73.8	4.64	2.05	.....	.....	Roots 42 hours in RbBr; shoots attached
2	1.0 – 2.0	91.0	3.28	1.70	.....	.....	
3	2.0 – 3.0	96.4	2.50	1.60	.....	.....	
4	3.0 – 4.0	94.2	1.50	1.50	.....	.....	
5	4.0 – 5.0	81.6	0.98	1.12	.....	.....	
1	0.0 – 1.0	128.6	4.11	0.41	0.53	1.64	42 hours in RbBr followed by 48 hours in tap water; shoots attached
2	1.0 – 2.0	57.1	1.08	0.09	2.20	1.61	
3	2.0 – 3.0	74.0	1.41	0.21	1.09	1.40	
4	3.0 – 4.0	89.5	1.25	0.35	0.25	1.15	
5	4.0 – 5.0	93.5	1.03	0.56	.....	0.56	

* Milligram-equivalents $\times 10^{-3}$

Apparently the removal of rubidium and bromide is not equally distributed along the root. In total amount, as well as concentration, the most bromide was withdrawn by the shoot from the apex and the least from the more distant segments. The apical segments of the roots, however, which were attached to the shoot and which were in contact with rubidium bromide, grew by enlargement during period 2 and retained rubidium; although the

fall in *concentration* was greatest in the apical section, the *absolute amount* removed was actually greatest from the next older segment. Data of period 3 yielded the same conclusion.

The conclusion from these data is, therefore, that the growing regions of the root apex are not only the regions which are most able to accumulate the salts from the external solution but, in competition between the root and the shoot, salts are first removed from the segments near the apex for transfer to the shoot. It must not, however, be supposed that salt relatively far from the apex (up to 5 cm. in barley roots) is inaccesible to the shoot. Even at this distance where the endodermis is commonly suberized (**6**) and where new root initials may be present, the absorbed salt may move to the shoot under the influence of a deficit in the total supply. Absolute proof is lacking that the drop in salt concentration and amount in the older segments of roots of series III (plants in tap water, after bromide) during periods 2 and 3 was really due to export to the shoot. However, the only conceivable alternative, namely that salt had passed out into the external solution from cells which were now in a senescent decline, presupposes a condition for which there is no evidence and which is inconsistent with the demonstrable intake of salt in series I by similar cells and, in series II, with their ability to retain salt previously accumulated even after the shoot was removed.

These results, therefore, so far as they go, emphasize that removal to the shoots proceeds most readily from the regions near the root tip. Entry into the stele from the cortical tissues presents many problems. As shown by investigations of the concentration of solutes in the liquid exuded from the stele of decapitated plants, the salts may finally appear (if the root is aerated) in the stelar fluids at concentrations greater than that in the external solution. It is reasonable to suppose that this, like accumulation in living cells, demands the activity of living tissues and it is significant that the region in the root from which salts are so readily withdrawn is one in which growth and tissue differentiation are still in progress.

It would be idle to deny that the details of the removal of salt from the root are still as obscure as when PREVOT and STEWARD pointed out that the "factors which in the intact plant cause this ready removal of electrolytes from root cells which have already accumulated them and which, in excised roots, would necessitate drastic treatment with perhaps irreversible changes represents one of the most difficult and fundamental of outstanding problems." Beyond emphasizing that the stimulus for removal of salt from the root is clearly transmitted from the shoot, little can be said concerning this mechanism. This, however, already implies a great deal, for the shoot also provides the stimulus for the continued growth of the root and only in so far as the growth of the apex is maintained would the root be expected to retain cells with the necessary degree of activity for the work of absorption,

accumulation, and secretion into the xylem of which it is clearly capable. After removal of salt from relatively near the root tip it also disappears from cells farther back. It seems more reasonable to suppose that salt is transferred in the cortex to that region of most active transfer to the shoot than to postulate that it passes across an older endodermis which is suberized. Thus, in barley, salt contained in virtually the whole of the root cortex becomes accessible to the shoot since the new growing centers of lateral roots in the still older regions are active in absorption and transfer and they pierce what would otherwise be an impermeable barrier of endodermis without passage cells.

Summary

There is in barley roots a longitudinal gradation in the accumulation of cations similar to that already demonstrated for an anion (Br).

The gradation in accumulation has been demonstrated for rubidium and potassium by the use of spectrographic methods.

Roots attached to the plant and in contact with a salt solution, like excised roots, show a gradation along their length of salt concentration—the greatest concentrations being at the apex.

Barley plants, grown without access to external salts, will accumulate in their roots a greater concentration of rubidium than of bromide from aerated rubidium bromide solutions. The leafy shoot is the principal region of bromide accumulation; the average concentration attained may be in excess of that even in the root tip. Bromide moves to the shoot more readily than rubidium.

After contact with rubidium bromide solutions, and if transferred to solutions free of this salt, the shoot depletes the root of the salts it had previously absorbed—especially the bromide.

When the shoot removes rubidium and bromide from the root it does so first from the younger segments. Eventually, however, ions—especially bromide—may be withdrawn even from levels at which the endodermis should be impermeable. During this process the normal gradient of salt accumulation may be reversed.

It is in the region of the root in which growth and vascular differentiation occur that transfer from the storage cells of the root to the stele for movement to the shoot is localized.

Accumulation of rubidium does not progress for long in excised roots which lack the stimulus of the shoot; in the attached roots, however, despite some removal to the shoot, the concentrations continue to increase for a long period (at least to 130 hours).

The shoot, which provides the stimulus for removal of salt from the root also, by promoting the continued growth of the root, maintains at its apex the development and activity of the tissues chiefly responsible for absorbing

salt from the external solution and transferring it to the stele and thence to the shoot.

The uptake and distribution of salt is, therefore, responsive to the factors which in the whole organism correlate and integrate the growth of its parts.

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SULPHUR CONTENT OF SEEDS AND SEED WEIGHT IN RELATION TO EFFECTS OF SULPHUR DEFICIENCY ON GROWTH OF SUNFLOWER PLANTS

SCOTT V. EATON

(WITH FIVE FIGURES)

Introduction

Detailed studies have previously been made by the writer on the effects of sulphur deficiency on the growth and metabolism of two plants, the soy bean (**6**) and the sunflower (**7**). No sulphur was intentionally added to the nutrient solution used. Yet considerable growth was made by each plant. The minus-sulphur soybean plants weighed about 70 per cent. as much as the plus-sulphur. Sulphur deficiency reduced the weight of the stems of the sunflower plants to 28 per cent. of the weight of the controls. These data show that the plants were getting considerable sulphur as an impurity.

The main possible sources of this sulphur were: The sulphur in the air, part of which dissolved in the moist sand (**1**) and was absorbed by the roots of the plants or taken in by the plants directly from the air (**16**); the sulphur in the salts in the nutrient solution; and the sulphur in the seed. When plants are grown in the open there is an additional source of sulphur, the sulphur brought down by the rain (**1, 8**).

The object of this work was to study the sulphur content of sunflower seeds as a sulphur impurity. It was thought that if the plants were grown to maturity with a minus-sulphur nutrient solution, the seeds produced would have a lower sulphur content than the seeds of plants grown with complete nutrient. By using the low-sulphur seeds it should be possible to get greater effects of sulphur deficiency than with high-sulphur seeds.

As will be described presently, however, the percentage sulphur of the seeds of the sulphur-deficient plants was the same as the seeds of the plus-sulphur plants. But the former seeds were much smaller than the latter so that the problem resolved itself partly into a study of the effects of the size of the seed on the growth of the plant. The less actual sulphur present in the smaller seeds of the sulphur-deficient plants was of course one factor affecting the size of the plants produced by these seeds.

Methods

The minus-sulphur and plus-sulphur plants were grown to maturity in quartz sand in the greenhouse in the spring and summer of 1939. They were part of the same crop used in the study of the effects of sulphur deficiency on the metabolism of the sunflower (**7**). The methods of growing the plants are

described in this paper. The plants were harvested when they were dead, and the heads and fruit dry. The harvest period of the minus-sulphur plants extended from July 11 to August 3; of the plus-sulphur from July 11 to August 11. The heads were cut, put into paper sacks, and later the fruits were removed from the heads and the seeds hulled out.

Samples of the minus-sulphur seeds, the plus-sulphur seeds and also of the original seeds used in producing these two lots of seed were analyzed for sulphur by the official sodium peroxide method (**3**). Great care had to be

FIG. 1. Plus-sulphur and minus-sulphur plants that were used to produce the corresponding lots of seeds, 39 days from the time of planting the seeds.

used to prevent ignition of the material. The high fat content no doubt favored ignition. Attempts to use the Parr bomb method failed probably because the material was rather coarsely ground. It was impossible to grind the seeds to a fine state because of the high fat content.

During the spring of the next year, 1940, plants were grown from seeds of each of the above three lots. The seeds were selected for uniformity and the larger, better formed seeds were used for planting. There were 40 plants in each lot, half of which were grown with a complete nutrient solution and half with a solution lacking sulphur. The plan of growing the plants was the same as that used in the previous year. The seeds were planted April 6, and the plants harvested May 18. The growth period was thus six weeks.

Data

Figure 1 shows the appearance of the plants that were used to produce the plus-sulphur and minus-sulphur seeds. The photograph was taken just before some of the plants had formed small flower heads. It shows well the differences in height, size of leaves, and thickness of stems of the plants of the two lots but it does not do justice to the difference in color. The minus-sulphur plants were much lighter green than the plus-sulphur. Figure 2

FIG. 2. Plants of the same lots as those of figure 1 at a later stage when most of the plants had blossomed, 91 days from the time of planting the seed. Plus-sulphur on left, minus-sulphur on right.

shows the plants at a later stage when most of them had blossomed. The relative height of the plants and size of the heads are shown. Figure 3 is a close-up of a representative minus-sulphur and plus-sulphur flower head showing the relative size.

Table I gives data for the number, weight, and sulphur content of the minus-sulphur and plus-sulphur seeds, including also data for the original seeds that were used in producing the seeds of these two lots. The minus-

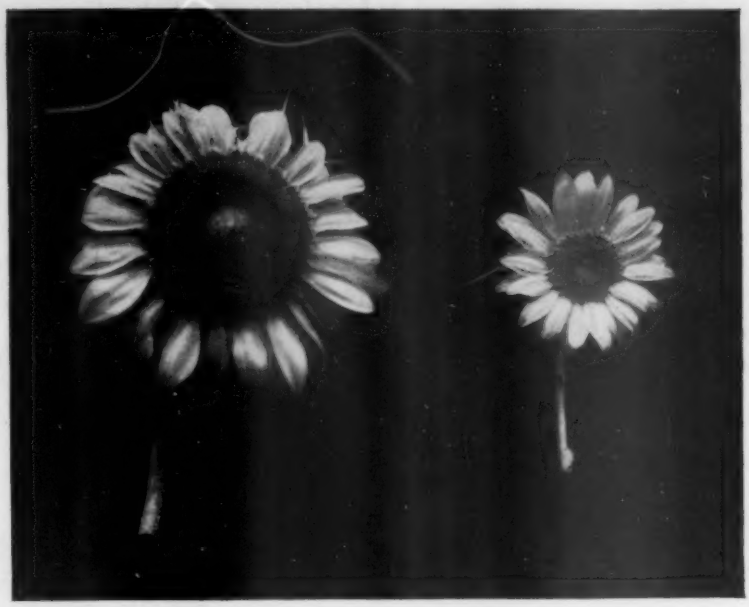

FIG. 3. Close-up of flower heads of the plants of figure 2, plus-sulphur on left, minus-sulphur on right.

sulphur seeds were fewer in number and weighed much less than the plus-sulphur. On the other hand, the sulphur content of the former seeds was exactly the same as the latter on a percentage basis, although much less in amount per seed because of the smaller size. The plus-sulphur seeds which were produced with a complete nutrient solution were not as large as the original seeds, but they contain a higher percentage of sulphur. The actual

TABLE I

NUMBER AND WEIGHT OF SEEDS, SULPHUR CONTENT

SERIES*	AVERAGE NUMBER OF SEEDS PER HEAD	WEIGHT OF SEEDS HARVESTED (GM. PER 100 SEEDS)	WEIGHT OF SEEDS PLANTED (GM. PER 100 SEEDS)	SULPHUR (PERCENTAGE DRY WEIGHT BASIS)	SULPHUR (MG. PER 100 SEEDS)
		gm.	*gm.*	%	*mg.*
MS	15.1	1.650	2.260	0.480	10.8
PS	164.2	3.705	5.280	0.480	25.3
OS	.....	5.880	6.420	0.405	26.0

* MS, PS, and OS refer respectively to the seeds of plants grown with a minus-sulphur nutrient solution, with a complete nutrient solution, and the original seeds used in producing these two lots.

amount of sulphur present per seed, however, was slightly less because of the smaller seeds. Since the larger better formed seeds were selected for planting, the weight of the seeds planted was greater for each lot than that of the seeds harvested.

Figures 1 and 2 show that the plants producing the minus-sulphur seeds were much smaller than those producing the plus-sulphur seeds; table I shows that the former seeds weighed much less than the latter. Yet the minus-sulphur seeds gave practically as good germination as the plus-sulphur and slightly better than the original seeds. The percentages of germination (as determined by the percentage of seed planted that produced plants) were

FIG. 4. Minus-sulphur plants produced by the three lots of seeds. From left to right: plants produced by minus-sulphur, by the original, and by plus-sulphur seeds.

respectively for the three lots of seeds, minus-sulphur, plus-sulphur, and the original seeds: 98.0, 98.7, and 97.5 per cent.

Figure 4 shows the appearance of the minus-sulphur plants that were produced from the three lots of seeds, figure 5 the plus-sulphur plants. The plants shown in each figure were the same distance from the camera so that the relative size is seen by comparing the plants in corresponding lots. In each case the plants developing from the plus-sulphur seeds are the largest, those developing from the minus-sulphur seeds the smallest, and the plants produced by the original seeds intermediate in size. The intermediate position of the last-named plants in the case of those grown with complete nutrient is not very evident in the photograph, however, because the plants bend over somewhat and one of the plants of the group developing from the minus-

sulphur seeds is especially tall. The typical symptoms of sulphur deficiency in the sun-flower: yellow-green color of the leaves, smaller leaves, and thinner stems, which have been noted in previous work (**8**) may be seen by comparing plants of corresponding lots in the two figures.

Table II gives the data for weight, height, and top-root ratios of the plants that developed from the minus-sulphur and plus-sulphur seeds. There are included also data for the plants produced by the original seeds. The figures in bold face type express the data on a relative basis and are referred to later. It is to be noted that whether the plants are grown with sulphur or without

FIG. 5. Plus-sulphur plants produced by the three lots of seeds. From left to right: plants produced by minus-sulphur, by the original, and by plus-sulphur seeds.

sulphur, those produced by the heavier plus-sulphur seeds are larger and taller than the plants developing from the smaller minus-sulphur seeds. This is true of the entire plant and each of its parts. The plants that developed from the original seeds are intermediate in height and weight between the plants of these two lots.

The top-root ratios vary inversely with the size of seeds used in producing the plants, indicating that decreasing the size of the seeds affects the roots more than the tops. There is a regular decrease in the size of the seeds beginning with the original seed. On the other hand, there is a regular increase in the top-root ratios beginning with the plants produced by these seeds, no matter whether the plants are grown with the complete nutrient solution or with one lacking sulphur. As may be seen by compar-

ing the top-root ratios for plants grown with sulphur with the ratios for corresponding lots of plants grown without sulphur, sulphur deficiency affects the tops more than the roots.

By comparing the weights of plants grown with a complete nutrient solution with the weight of corresponding lots of plants grown without sulphur it is seen that sulphur deficiency greatly decreases the weight of the entire plant and each of its parts. This comparison was put on a percentage basis for the entire plant by dividing the weight of the minus-sulphur plants by the weight of the corresponding plus-sulphur plants.

TABLE II

GREEN WEIGHT (GM. PER 20 PLANTS), AVERAGE HEIGHT PER PLANT, TOP/ROOT RATIO, WEIGHT OF SEEDS

SERIES	LEAVES	STEMS	TOPS	ROOTS	ENTIRE PLANT	TOP/ ROOT RATIO	HEIGHT	SEEDS
	gm.	*gm.*	*gm.*	*gm.*	*gm.*		*in.*	*gm.*
MS*								
−S	141.700	229.700	371.400	49.200	420.600	7.548	18.520	2.260
	1.000†	**1.000**	**1.000**	**1.000**	**1.000**		**1.000**	**1.000**
PS								
−S	221.500	490.000	711.500	109.300	820.800	6.509	26.570	5.280
	1.533	**2.133**	**1.915**	**2.221**	**1.951**		**1.434**	**2.336**
OS								
−S	175.700	313.300	489.000	96.500	585.500	5.067	22.540	6.420
	1.239	**1.363**	**1.316**	**1.949**	**1.392**		**1.217**	**2.840**
MS								
+S	490.520	1137.000	1627.520	95.880	1723.400	16.974	38.600	2.260
	1.000	**1.000**	**1.000**	**1.000**	**1.000**		**1.000**	**1.000**
PS								
+S	751.700	1810.700	2562.400	195.000	2757.400	13.140	44.420	5.280
	1.532	**1.592**	**1.574**	**2.033**	**1.599**		**1.150**	**2.336**
OS								
+S	671.400	1395.700	2067.100	190.100	2257.200	10.873	40.400	6.420
	1.368	**1.227**	**1.270**	**1.982**	**1.309**		**1.046**	**2.840**

* MS, PS, and OS have the same meaning as in table I; −S means that the plants were grown without sulphur in the nutrient solution; +S that sulphur was present.

† The figures in bold face type express the data on a relative basis.

Thus it was found that in the three lots of plants those grown from the minus-sulphur seeds, those from the plus-sulphur, and those from the original seeds, the minus-sulphur plants weighed only 24.4 per cent. as much as the plus-sulphur in the first, 29.7 per cent. in the second and 25.9 per cent. in the third. So sulphur deficiency has the greatest effect in reducing the growth of the plants in the case of the small minus-sulphur plants which contained the least sulphur, calculated on an absolute basis (table I). The differences are small, however.

In table II the data are also expressed on a relative basis. While the plants that developed from the small, minus-sulphur seeds weigh less and

are shorter than those produced by the larger plus-sulphur seeds, in proportion to the size of the seeds the former plants are larger and taller than the latter. In other words, the plants developing from the small seeds tend, during their growth period, to gain in height and weight on the plants which developed from the large seeds. For example, the ratio of the weight of the seeds of these two lots is 1.000 : 2.336; the ratio of the weights of the entire plant is 1.000 : 1.951 when the plants are grown without sulphur; and only 1.000 : 1.599 when they are grown with sulphur present. A similar relationship holds for the weight of the tops, leaves, and stems and the height of the plant, but the ratio for the weight of the roots is much closer to that of the weight of the seeds than is true of the other ratios. As stated above, decreasing the size of the seeds affects the roots more than the tops.

While the original seeds are larger than either of the other lots, the plants produced by these seeds are inferior in weight and height to the plants developing from the smaller plus-sulphur seeds although superior to the plants produced by the minus-sulphur seeds.

Discussion

It is conceivable that when plants are grown to maturity in minus-sulphur solutions, one of two responses may be secured relative to the size of seeds and their sulphur content. On the one hand, the seeds may be smaller but have about the same percentage sulphur content as the seeds of plants grown with the complete nutrient; on the other hand, larger seeds than in the first case but of lower sulphur content may be produced. In the present work the minus-sulphur seeds were much smaller than the plus-sulphur, but they had exactly the same percentage sulphur content although they contained, of course, a much lower amount of sulphur figured on the absolute basis. In the tomato, however, NIGHTINGALE (**12**) found that the seeds of ripe fruits of plants grown without sulphur were much lower in the percentage of sulphur than those of plants grown with a complete nutrient. He does not give the weight of the seeds nor does he use the seeds in growing a second generation of plants.

A somewhat greater reduction in the growth of plants due to sulphur deficiency was obtained in this experiment by using the seeds of plants grown to maturity without sulphur, than with the seed of plus-sulphur plants. As stated above, the former seeds contain much less sulphur per seed than the latter. But the reduction in growth was not much greater for the minus-sulphur seeds. As a means of reducing the error due to the sulphur impurity in the seeds, the method is unsatisfactory because of the smaller seeds and the smaller plants which these seeds produce. Anyway, probably other sources of error, for example the sulphur of the air dissolving in the moist sand (**1**) and the sulphur in the nutrient salts, are more important.

The effect of the size of the seed in the growth of the plant is clearly shown in this work. Considering merely the two lots of seeds, the minus-sulphur and the plus-sulphur, since we know nothing about the conditions under which the original seeds were produced, the plants produced by the small, minus-sulphur seeds weigh much less than those developing from the large, plus-sulphur seeds (table II). This is true no matter whether the plants are grown with a complete nutrient solution or with a solution lacking sulphur. These results are in accord with much of the data in the literature. KIESSELBACH (**9**) summarizes the results of the long-time Nebraska experiments on the effects of the size of the seed on the yield of the small grain crops. The results are uniformly in favor of the larger seeds although the differences in favor of these seeds vary with the spacing and are quite small in the case of unselected seeds. MCCOMB (**11**) found that the weight and height of chestnut oak seedlings were directly correlated with the weight of the acorn planted. A similar relationship has been noted in plants that are propagated vegetatively. APPLEMAN (**2**) found in the potato that as the size of the seed piece was decreased below a certain minimum, the vigor of the sprout decreased; and also that within certain limits the yield of tubers was directly correlated with the size of the seed piece.

While the size of the plant is usually directly correlated with the size of the seed, large seeds may not produce plants proportionally larger as compared with small seeds. TRELEASE and TRELEASE (**17**) found that the dry weight of the tops and roots of wheat plants was directly related to the size of the seeds but that per unit of seed weight the plants developing from the small seeds were larger than those developing from the large seeds. In the present work also this relationship held. The plants produced by the small minus-sulphur seeds gained on those developing from the larger plus-sulphur seeds, so that at the time of harvest the former plants were, relative to the sizs of the seeds, larger than the latter. This relationship was more pronounced when the plants were grown with a complete nutrient solution than when grown with a solution lacking sulphur (table II).

The larger size of plants produced by heavier seeds may not persist to maturity. KOTOWSKI (**10**) found that the effect of the size of the seed in the case of the pea and the broad bean was evident early in growth period but that it disappeared before blooming and did not affect the weight of seed produced. Also, the yield of cabbage heads was not affected by the size of the seed. The plants of the present experiment were harvested when a few of them had developed small flower heads. But the influence of seed size on the size of plants was so marked at this time that the effect would probably have persisted to maturity and have affected seed production.

Usually heavier seeds produce larger plants. But sometimes the largest plants develop not from the heaviest seeds but from seeds of an intermediate

weight. For example, SCHMIDT (**15**) found that the largest buckwheat plants developed from seeds of high medium weight (next to the heaviest of five weight grades) rather than from abnormally heavy seeds, although with this exception the size of plant corresponded with the size of seed. ROTUNO (**13**) found that while there was quite a variation in different varieties of radishes, on the average the medium weight seeds produced the heaviest roots. In the present work too, if we compare the three lots, the medium weight seeds produced the largest plants. The plants developing from the plus-sulphur seeds were larger than those produced by the smaller, minus-sulphur seeds or the larger original seeds used in producing these two lots of seeds (table II). This lack of correlation between size of seed and plant produced by it is probably due to a difference in chemical composition which resulted from the difference in the conditions under which the three lots of seeds were produced. This is referred to again below.

The larger plants produced by heavier seeds are no doubt due to the greater food reserves of the seed. The influence of these food reserves is better shown when the complicating effects of photosynthesis are excluded. RUDOLFS (**14**) grew bean seedlings in the dark at different temperatures. Growth rate curves are drawn. The early increasingly rapid growth and the slowing up of growth as the food reserves become exhausted are well shown. Throughout the growth period the seedlings produced by the large seed were larger than those developing from the small seed. Growth at higher temperatures increased this advantage, the temperature coefficient for the larger seeds being normal and larger than that for the smaller seeds. The seedlings developing from the small seeds had an abnormally low temperature coefficient.

When the plants are grown in light, before the slowing up of the growth rate due to the exhaustion of the food reserves of the seed the leaves are present and are making food materials by photosynthesis more rapidly than they were being provided by the digestion of the seed reserves. So the growth rate does not fall off but continues to increase until the final reduction due to seed production. The normal growth curve is the sigmoid curve (**4**). The larger seeds produce larger plants with greater leaf area so that these plants keep ahead of the smaller plants developing from the lighter seeds.

It would take a detailed analysis to explain on the basis of the seed reserves the differences in the size of plants produced by seeds of differing weights. The only compound determined in the present work was sulphur. The greater actual sulphur content of the plus-sulphur seeds was certainly one reason for the larger plants produced by these seeds in comparison with the minus-sulphur seeds. But there were no doubt other decided differences in the chemical composition of the two lots, for plants grown in minus-sul-

phur solutions have been found to have a decidedly different chemical composition than plants grown in a complete nutrient solution (**6, 7, 12**).

Though the original seeds were larger and contained on an absolute basis slightly more sulphur than the plus-sulphur seeds, they produced smaller plants than did the latter. The original seeds were from a commercial lot and nothing is known as to the conditions under which they were grown. But the plants were no doubt grown in soil. The plus-sulphur seeds were produced by plants growing in sand and supplied at all times with an abundance of the various essential elements. Seeds produced under these conditions will probably have more of various mineral and organic compounds than those produced by plants growing in soil perhaps deficient in one or more of the sesential elements.

Chemical composition is no doubt the main reason for the relationship between size of seed and size of plant. But another factor that must be considered is competition among plants. This may help explain cases of lack of close correlation between weight of seed and weight of plant. If the planting of the small and large seeds is at the same rate and too close for the large seeds, then the smaller plants developing from the small seeds will interfere less with each other's growth than will the larger plants so that, relative to the size of seeds, their growth will be greater. In the present work the seeds of the three lots were planted at the same rate. So competition among plants may be one factor in accounting for the facts that the seeds of intermediate weight, the plus-sulphur seeds, produced the largest plants and that the small minus-sulphur seeds produced relatively larger plants than the larger plus-sulphur seeds. Also, cases reported in the literature of the failure of larger plants, developing from heavier seed, to maintain their advantage to maturity may be partly explained by competition. CLEMENTS, WEAVER and HANSON (**5**) are the authors of an excellent monograph on plant competition.

The effect of weight of seed on the size of plant produced emphasizes the importance of seed selection in research work. If seeds are selected for uniformity, the individual plant differences will be much smaller than with unselected seeds, and fewer plants will be required to secure significant differences by certain variations in the nutrient solution or other treatment of plants. For example, TRELEASE and TRELEASE (**17**) show that 3.95 times as many wheat plants of the unselected group would be needed for a certain degree of accuracy as would be needed of the group the seeds of which were selected to a 4-mg. range of variation.

Summary

1. An attempt was made to reduce the sulphur content of their seeds by growing sunflower plants to maturity with a nutrient solution lacking sul-

phur. Control plants were grown to maturity with a complete nutrient solution. The minus-sulphur and plus-sulphur seeds and also the original seeds used in producing these two lots were used in studying the effect of sulphur deficiency and seed weight on the growth of the plants.

2. The minus-sulphur seeds were much fewer in number per head and much smaller than the plus-sulphur seeds. But they contained the same percentage sulphur as the latter, although much less, of course, on an absolute basis. The plus-sulphur seeds were smaller than the originals. They contained slightly less actual sulphur per seed than the originals but more on a percentage basis.

3. The small minus-sulphur seeds germinated practically as well as the larger plus-sulphur and slightly better than the original seeds. The percentage germination of the first was 98.0 per cent., of the second 98.7 per cent. and of the original seed 97.5 per cent.

4. Sulphur deficiency reduced the size of the plants produced by the minus-sulphur seeds more than that of the plants developing from the plus-sulphur seeds. The difference was small, however. As a means of reducing the error due to the sulphur impurity in the seeds, the method is unsatisfactory because of the relatively small plants produced by the small minus-sulphur seeds.

5. The effect of seed weight on the size of the plant was clearly shown. The plants developing from the small minus-sulphur seeds were much smaller than those produced by the larger plus-sulphur seeds. Considering the three lots of seeds, however, the plus-sulphur which were intermediate in weight between the minus-sulphur and the originals produced the largest plants.

6. Relative to the size of the seeds, the plants developing from the minus-sulphur seeds were larger than the plants produced by the plus-sulphur seeds. This advantage was greater when the plants were grown with sulphur than when they were grown without sulphur in the nutrient solution.

7. As shown by the top-root ratios, size of seed affected the root more than the top; sulphur deficiency affected the top more than the root.

8. The larger plants produced by heavier seeds is due mainly to the greater amount of storage compounds in the seed. The only compound determined in the present work was sulphur. The greater amount of sulphur in the larger plus-sulphur seeds was certainly one reason for the larger plants produced by these in comparison with the minus-sulphur seeds. It is not known why the original seeds which were larger and contained slightly more sulphur than the plus-sulphur seeds did not produce as large plants.

9. Competition among plants may account partly for the results obtained in the present work.

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EFFECT OF ORCHARD FERTILIZER APPLICATIONS ON THE COMPOSITION OF APPLES[1]

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Studies of the maturity of a number of varieties of fruit have been in progress at the Washington Station over a period of years. The effect on tree response of different fertilizer treatments applied to Jonathan apple trees, as indicated by a number of types of measurements, has also been determined. A number of reports on these latter experiments have been made. During the simultaneous progress of the maturity and fertilizer studies, a study of the effect of different fertilizers on the chemical composition of the fruit has been made. A major part of this latter phase of the work has been done on Jonathan apples; information has also been collected on the mineral composition of Winesap apples.

Brown (**1**) reported that the ash, potash, phosphate, and phosphate-nitrogen ratio in apples were highest when the trees were growing upon a silt and gravel soil, lowest on the fen and fine silt soils, and intermediate on chalk and sandstone. DeLong (**2**) reported that, upon the basis of the percentage of the individual ash constituents in fresh Wagener apples grown under Nova Scotia conditions, phosphorus and potash were lowest in fruits from the nitrogen fertilized trees; and that applications of muriate of potash materially increased ash and potash contents of the apples.

The preceding reports indicate that the fertilizers applied might have influenced the mineral content of the apples. Studies are reported herewith of the effect of orchard fertilizer applications upon the chemical composition of Jonathan and Winesap apples.

Experimental procedure

Samples of fruit were collected from the different fertilizer plots of the Washington Agricultural Experiment Station in North Central Washington at intervals over a period of six years.

The plots of Jonathan apple trees were located on Ephrata fine sandy loam in the orchard of Frank Von Osten in the East Wenatchee district. Nitrogen was applied annually from 1929 to 1936, inclusive, in the form of ammonium sulphate to supply amounts of actual nitrogen equivalent to one pound per tree. Phosphorus was applied in the form of superphosphate at the rate that would supply one pound per tree. Potassium was applied in the form of potassium chloride to supply amounts of actual K_2O totaling 2.5 pounds per tree.

[1] Published as Scientific Paper no. 524, College of Agriculture, and Agricultural Experiment Station, State College of Washington, Pullman, Washington.

The Winesap plots were located on Cashmere coarse sandy loam at the Tree Fruit Branch Experiment Station near Wenatchee. Nitrogen fertilizer was applied in the form of ammonium sulphate and sodium nitrate in amounts to supply equal quantities of actual nitrogen totaling one pound per tree annually from 1929 to 1935, inclusive. After 1935 the actual nitrogen applied annually was reduced to 0.6 pound per tree. Potassium was applied in the form of potassium chloride and potassium sulphate to supply equal amounts of K_2O at the rate of two pounds per tree. Phosphorus was applied to soil under the Winesap trees in the same form and quantity as for the Jonathan.

With each variety the fertilizers were broadcast on the ground uniformly over the entire plot area, usually some time in February. The trees were planted 26 feet apart on the diagonal and were approximately 25 years old when the experiments were started in 1929.

Methods of analysis

In preparing samples for analysis at least 25 apples were peeled, quartered, cored, and thin slices taken from each quarter until a sample of the required weight was obtained.

In determining the dry weight, 50 grams of the sample as previously prepared were dried on a steam bath and then in a vacuum oven at 15 cm. at 70° C. for five hours.

The sugar and the acid-hydrolyzable reducing material were determined by extracting 25 grams of a fresh sample with 95 per cent. alcohol for 12 hours. The extract was made up to volume and the reducing substances were determined on a 10-ml. aliquot by the MUNSON and WALKER method, weighing the cuprous oxide; reducing sugars were calculated from this determination. Sugars in 50 ml. of the alcoholic extract were hydrolyzed by adding 10 ml. of hydrochloric acid, making to volume, and allowing to stand overnight; reducing substances were again determined as previously indicated and calculated as dextrose. The residue from the alcoholic extract was hydrolyzed with 125 ml. of 2.5 per cent. hydrochloric acid under a reflux condenser for two and one-half hours. The solution was neutralized with sodium hydroxide, made to volume, and the reducing substances determined on a suitable aliquot and calculated as dextrose. This fraction is referred to as alcohol-insoluble acid-hydrolyzable material. This method of determination of sugars was utilized in obtaining the data in tables I and III.

In obtaining the data for sugars presented in table IV, the fruit was frozen and the juice expressed according to the methods described by ST. JOHN and VAN AMBURGH (9). Final determination of the different fractions of reducing sugars obtained in the juice was made by the LANE-EYNON titration method for reducing sugars as described in later editions of the

A.O.A.C. methods. These methods are more fully described in papers by ST. JOHN and MORRIS (8) and ST. JOHN and VAN AMBURGH (9).

Acid in the fruit was determined by making a water extract of a weighed sample and diluting to 500 ml. Toluene was added. After standing with frequent shaking for three days, an aliquot of 100 ml. was titrated with 0.1 N sodium hydroxide using phenolphthalein as the indicator.

Ash was determined by igniting in a muffle furnace the sample remaining from the determination of moisture; pH of the juice was obtained by utilization of a pH meter with glass electrode.

Nitrogen was determined by the Kjeldahl method using a 25-gram sample prepared according to the method previously described. The results for the elements calcium, phosphorus, and potassium were obtained by A.O.A.C. methods (1930) under the section presenting procedure utilized in analyzing plant material.

Results

MINERAL ELEMENTS AND NITROGEN

The results of analyses of fruits of Jonathan apples of the 1931 crop, showing the percentage of nitrogen, ash, and mineral elements are presented in table I. This includes data on samples of fruit analyzed immediately after harvest and again after the fruit had remained in storage at 32° F. until the following March.

TABLE I

MINERAL COMPOSITION OF JONATHAN APPLES AS INFLUENCED BY FERTILIZERS. 1931 HARVEST. EXPRESSED AS PERCENTAGE OF FRESH FLESH WEIGHT

FERTILIZER ADDED	CONTROL	NPK	NPK	NP	NK	PK	N
ANALYZED IMMEDIATELY AFTER HARVEST							
	%	%	%	%	%	%	%
Nitrogen as N	.....	0.03	0.02	0.04	0.05	0.03	0.05
Phosphorus as P	.....	0.009	0.008	0.007	0.008	0.008	0.008
Potassium as K	.....	0.124	0.106	0.108	0.105	0.112	0.099
Calcium as Ca	.....	0.005	0.005	0.005	0.005	0.005	0.005
Ash	.....	0.25	0.23	0.25	0.25	0.26	0.26
ANALYZED AFTER STORAGE AT 32° F. UNTIL MARCH							
Nitrogen as N	0.03	0.02	0.03	.....	0.03	0.03	0.02
Ash	0.3	0.26	0.25	.....	0.26	0.3	0.25

While there was a slight variation in the percentage of nitrogen in the fruit, this did not appear to be definitely related to the use of nitrogen in the fertilizer applied. The percentage of phosphorus in the fruit from the different plots varied only slightly, and the same was true of potassium

and calcium. Thus, the amount of these elements in the fruit did not appear to be definitely influenced by the fertilizer applications to the soil.

In 1933 samples were collected from the plots of Jonathan apples receiving fertilizer applications as previously described. Collections were also made from two additional plots, one of which had received an application of phosphorus alone and the other an application of potassium alone, each in amounts per tree comparable to those applied when used in combination. In addition, Winesap apples were collected from the plots that had received fertilizer treatments as first described. Data showing the nitrogen, phosphorus, potassium, and total ash of both varieties of apples produced during the 1933 season appear in table II.

TABLE II

MINERAL COMPOSITION OF APPLES AS RELATED TO FERTILIZER TREATMENTS. CROP OF 1933. EXPRESSED ON ORIGINAL SAMPLE BASIS AS PERCENTAGE OF FRESH FLESH WEIGHT

FERTILIZERS APPLIED	CONTROL	NPK	NP	NK	PK	N	P	K
	JONATHAN							
	%	%	%	%	%	%	%	%
Nitrogen as N	0.02	0.03	0.02	0.03	0.03	0.02	0.02	0.02
Phosphorus as P	0.011	0.009	0.008	0.008	0.011	0.01	0.014	0.013
Potassium as K	0.068	0.075	0.055	0.063	0.072	0.06	0.075	0.077
Ash	0.25	0.28	0.2	0.22	0.26	0.22	0.26	0.26
	WINESAP							
Nitrogen as N	0.02	0.02	0.03	0.03	0.03	0.03	.....	.....
Phosphorus as P	0.012	0.009	0.01	0.009	0.012	0.01	.....	.....
Potassium as K	0.075	0.073	0.074	0.071	0.076	0.075	.....	.....
Ash	0.3	0.27	0.29	0.26	0.27	0.27	.....	.....

NITROGEN.—The data do not show with either the Jonathan or Winesap apples any consistent relationship between the percentage of nitrogen in the fruit and the fertilizers applied. These results do not necessarily mean that no more nitrogen was taken up by the tree when nitrogen was applied than when it was not added in the fertilizer. With respect to growth and fruit production, the first limiting major element in the soils of apple orchards of Central Washington is nitrogen (**6**). Hence, when nitrogen is applied, the increased growth and greater production of fruit may so utilize the larger nitrogen intake that the actual percentage in the apples is not consistently higher than without nitrogen applications, which results in lessened growth and production. For example, data show the greatest average annual terminal growth results from the applications of nitrogen alone, and the next greatest from the nitrogen and phosphorus applications in the orchard (**7**). Hence the percentage of nitrogen in apples from trees receiv-

ing nitrogen alone and from trees receiving nitrogen and phosphorus fertilizers was the same as that in the fruits from trees receiving phosphorus, those receiving potassium, and from the control trees.

With fruit from trees of the NPK and the NK plots the slight increase in the percentage of nitrogen found in the apples may be explained upon the basis of shorter annual terminal growth (NPK, 6.8 inches; NK, 6.3 inches) than that made by the trees of the N (9.8 inches) and NP (7.6 inches) plots, nitrogen having been applied in each case. The fact that the apples from the PK plot contained slightly more nitrogen than those from the control plot might again be explained upon the basis of difference in average terminal growth, 4.3 inches and 5.1 inches, respectively.

PHOSPHORUS.—The data show that where nitrogen was included in the fertilizers applied the percentage of phosphorus in the apples tended to be lower than when nitrogen was omitted. This was true even when phosphorus was added with the nitrogen. Furthermore, the percentage of phosphorus in the apples tended to be higher in fruit from the control trees receiving no fertilizers, than was found in fruits from trees receiving nitrogen alone or with phosphorus. This again appears to be related to the increased growth and greater production of fruit resulting from the nitrogen application (**7**). This response would tend to lessen the actual percentage of phosphorus in the fruit notwithstanding a greater possible total intake by the tree.

The trees receiving phosphorus alone produced fruits containing the highest percentage of this element in the apples. This may be explained upon the basis of both greater intake and reduced terminal growth as compared with the trees receiving nitrogen. The relatively high percentage of phosphorus in the apples from trees receiving potassium alone may be explained by the fact that these trees made the least annual terminal growth of any of the trees (1.6 inches).

POTASSIUM.—The percentage of potassium in the Jonathan apples seems again to be related to the greater annual terminal growth and fruit production resulting from the nitrogen applications. Where the growth and production were relatively small, the percentage of potassium was relatively high, regardless of whether potassium was included in the fertilizers applied. Its inclusion in the fertilizers, however, tended to increase the percentage in the fruits with or without nitrogen applications. The relatively high percentage of potassium in the apples receiving NPK as compared with the apples receiving NK may be explained by the relatively small production in 1933 of the trees in the NPK plot (556 bushels) as compared with the production per tree in the NK plot (847 bushels). The significance of the small difference in potassium may be questioned.

ASH.—DE LONG (**2**) studied the variability of the amount and com-

position of the ash of the Wagener apple under Nova Scotian conditions. The repeated applications of potassium greatly increased the ash content of the apples and the percentage of potassium in the ash. HOPKINS and GOURLEY (**4**) found that the application of fertilizers containing potassium resulted in an increase in the percentage of ash of apples and an increase in the amount of potassium in the ash of apples. This was especially true when phosphorus was omitted.

The data in tables I and II do not show any marked variation in the ash of Jonathan between the two seasons 1931 and 1933. DE LONG (**2**) reported, however, that the seasonal variations in ash of Wagener apples grown in Nova Scotia were large. Furthermore, the data in tables I and II do not show a definite difference in the ash between the Jonathan grown upon Ephrata fine sandy loam soil and the Winesap grown upon a Cashmere coarse sandy loam. The ash in these two varieties was somewhat less than that reported for the Grimes Golden in Ohio by HOPKINS and GOURLEY (**3**), which amounted to 0.42 per cent. The percentages of ash in the Jonathan apples show little definite relationship to the fertilizers applied. Further, the differences, in the percentages of the three major elements and of the ash in the Winesap apples are smaller and less definite than in the Jonathan.

ORGANIC CONSTITUENTS OF 1931 CROP

The percentage of dry matter, carbohydrates, acidity, and the pH value of the apples from the fertilizer plots were also determined. The data pertaining to the Jonathan apples of the 1931 crop, both shortly after harvest and following storage at 32° F. until March, are shown in table III.

DRY MATTER.—When the Jonathan apples were analyzed shortly after harvest, or after storage for nearly six months at 32° F., there seemed to be a slight tendency for fruit from the trees receiving nitrogen alone to be somewhat lower in dry matter than fruit from the other plots. In fact, with the exception of one sample from the NPK plot, all the fruits from trees receiving nitrogen even in combination with phosphorus or potassium or both, tended to be slightly lower in percentage of dry matter than did fruits without nitrogen, such as those from the PK plots.

When analyzed after about six months in storage at 32° F., the apples from trees receiving nitrogen alone or in combination, were always slightly lower in dry matter than fruit from trees receiving no nitrogen (control and PK trees).

ACIDITY.—The data showing the effect of the fertilizer applications upon the percentage of acid in Jonathan apples are not very conclusive. At the time of harvest, the percentage of acid in the Jonathan apples from trees receiving nitrogen alone was lower than in fruits from any of the other plots [(0.46 per cent.), except with one set of samples from trees receiving NPK (0.38 per cent.)].

After storage at 32° F. for nearly six months, Jonathan apples from trees receiving nitrogen either alone or in combination with P or K or both, were lower in acid than fruits from trees not receiving nitrogen (check and PK plots.)

SUGARS.—The possible influence of fertilizers upon the percentage of reducing sugars in Jonathan apples did not seem to be consistent. The percentage of sucrose, as well as total sugars, however, seemed to be highest in the Jonathan apples from trees receiving phosphorus and potassium without nitrogen. HOPKINS and GREVE (5) reported that applications of nitrogen to apple trees had little effect upon the percentage of carbohydrates in the fruit of Stayman Winesap apples.

TABLE III

INFLUENCE OF FERTILIZERS ON THE DRY MATTER, ACID, AND CARBOHYDRATES OF JONATHAN APPLES. CROP OF 1931. EXPRESSED AS PERCENTAGE FRESH FLESH WEIGHT

FERTILIZERS APPLIED	CONTROL	NPK	NPK	NP	NK	PK	N
ANALYZED SHORTLY AFTER HARVEST							
		%	%	%	%	%	%
Dry matter	.....	17.1	15.4	15.1	15.2	16.1	14.9
Acidity (as malic)	.....	0.55	0.38	0.6	0.64	0.58	0.46
Reducing sugars	.....	7.6	8.1	7.8	7.9	8.4	8.4
Sucrose	.....	1.6	0.9	1.6	2.3	3.8	2.8
Total sugars	.....	9.2	9.0	9.4	10.2	12.2	11.2
Alcohol insoluble acid-hydrolyzable material	.....	0.2	0.9	0.1	0.5	1.2	0.4
ANALYZED AFTER STORAGE AT 32° F. UNTIL MARCH							
Dry matter	17.1	14.2	15.6	.....	14.3	16.5	14.2
Acidity (as malic)	0.52	0.39	0.37	.....	0.41	0.42	0.39
Reducing sugars	6.4	7.9	6.5	.....	5.4	4.7	6.2
Sucrose	0.5	0.8	0.6	.....	1.6	5.0	0.9
Total sugars	6.9	8.7	7.1	.....	7.0	9.7	7.1
Alcohol insoluble acid-hydrolyzable material	0.7	0.2	0.4	.....	0.2	0.4	0.4

ALCOHOL-INSOLUBLE, ACID-HYDROLYZABLE FRACTION.—Upon the basis of the data in table III, it would appear that the percentage of alcohol-insoluble, acid-hydrolyzable fraction was highest in Jonathan apples from trees to which no nitrogen had been applied (control and PK). The percentage of this fraction was especially high shortly after harvest in fruit from the PK plots.

CROP OF 1933

Jonathan and Winesap apples of the 1933 crop were also analyzed for percentage of dry matter; acid (as malic); reducing, sucrose, and total sugars; and alcohol-insoluble acid-hydrolyzable materials. These data are presented in table IV.

TABLE IV

INFLUENCE OF FERTILIZERS ON THE DRY MATTER, ACID, AND CARBOHYDRATES OF JONATHAN AND WINESAP APPLES. CROP OF 1933. EXPRESSED AS PERCENTAGE OF FRESH FLESH WEIGHT

FERTILIZERS APPLIED	CONTROL	NPK	NP	NK	PK	N	P	K
	JONATHAN							
	%	%	%	%	%	%	%	%
Dry matter	13.8	13.9	13.8	13.0	14.0	13.2	14.5	13.3
Acidity (as malic)	0.45	0.37	0.35	0.42	0.45	0.4	0.51	0.4
Reducing sugars	7.6	7.6	7.8	7.5	7.3	7.6	7.3	7.4
Sucrose	1.6	1.8	1.5	1.2	2.3	1.4	2.8	1.9
Total sugars	9.2	9.4	9.3	8.7	9.6	9.0	10.1	9.3
Alcohol insoluble acid-hydrolyzable material	0.4	0.4	0.4	0.3	0.5	0.5	0.5	0.3
	WINESAP							
Dry matter	15.9	15.2	15.0	15.4	15.6	15.4	.....	.....
Acidity (as malic)	0.66	0.55	0.62	0.6	0.64	0.58	.....	.....
Reducing sugars	9.0	8.7	8.7	8.7	8.7	9.0	.....	.....
Sucrose	1.7	2.1	1.6	1.5	1.7	0.8	.....	.....
Total sugars	10.7	10.8	10.3	10.2	10.4	9.8	.....	.....
Alcohol insoluble acid-hydrolyzable material	0.4	0.4	0.7	0.4	0.9	0.6	.....	.....

DRY MATTER.—Neither the Jonathan nor the Winesap fruit showed any consistent difference in the percentage of dry matter as a result of fertilizer applications.

ACIDITY.—The data in table IV show that the Winesap apples were somewhat more acid than the Jonathan. Exclusive of apples from the trees receiving phosphorus alone (in the case of Jonathan) fruit of both Jonathan and Winesap tended to be slightly higher in acid when harvested from trees receiving no fertilizers. With the exception of the Jonathan from trees receiving K only and the Winesap from trees receiving NK, fruits of both varieties were lower in acidity when harvested from trees receiving nitrogen alone or in combination than from trees receiving no nitrogen.

SUGARS.—Jonathan apples did not show any consistent variation in sugars, with a possible exception of the percentage of sucrose in the plots receiving phosphorus alone or together with potassium. Sucrose in the fruit from the two plots was slightly higher than in that from the other plots.

Referring to the data on Winesap apples presented in table IV, the slight variation in sucrose previously discussed for Jonathan apples was not found. The percentage of sucrose in the Winesap apples obtained from the nitrogen treated plot was slightly below that found in the Winesap from the other plots; this difference, however, may not have been significant. The percentage of total sugars in the Winesap apples was slightly higher than that in the Jonathan apples.

ALCOHOL-INSOLUBLE ACID-HYDROLYZABLE FRACTION.—There was a slightly higher percentage of this fraction with Winesap apples from trees receiving PK without nitrogen than from trees in the other plots. With the Jonathan this was not the case, since the fraction was as high in apples from trees of the nitrogen plot as that found in apples from the other plots.

CROPS OF 1936 AND 1937

The data in table V are based upon analyses of the expressed juice and show the percentage of the different sugars based upon the total flesh weight as follows: levulose, dextrose, sucrose, reducing sugars, and total sugars; the acidity and pH values are also indicated.

TABLE V

SUGARS EXPRESSED IN JUICE OF JONATHAN APPLES HARVESTED IN 1936 AND 1937. EXPRESSED AS PERCENTAGE OF FRESH FLESH WEIGHT

FERTILIZERS APPLIED	CONTROL	NPK	NP	NK	PK	N
PICKED SEPTEMBER 25, 1936						
Levulose (%)	5.3	5.8	5.8	5.7	6.1	5.9
Dextrose (%)	1.2	1.2	1.4	1.5	1.7	1.4
Reducing sugars (%)	6.6	7.0	7.1	7.1	7.9	7.3
Sucrose (%)	2.9	3.3	3.1	3.0	3.0	3.6
Total sugars (%)	9.4	10.3	10.3	10.1	10.9	11.0
Acidity*	18.5	20.0	18.8	20.2	20.8	23.6
pH of juice	3.4	3.6	3.4	3.4	3.4	3.6
PICKED SEPTEMBER 27, 1937						
Levulose (%)	5.0	5.0	.....	5.0	5.3	5.1
Dextrose (%)	2.4	2.2	.....	2.2	2.2	2.6
Reducing sugars (%)	7.4	7.2	.....	7.1	7.7	7.8
Sucrose (%)	2.1	1.6	.....	2.0	2.0	1.5
Total sugars (%)	9.6	8.9	.....	9.1	9.6	9.3
Acidity*	15.3	14.2	.....	13.3	16.0	12.0
pH of juice	3.4	3.4	.....	3.4	3.4	3.4

* Milliliters of 0.116 N NaOH.

SUGARS.—With the 1936 crops, each of the sugars was slightly lower in the fruit obtained from the check plot than that in the fruit obtained from any of the fertilized plots. This was not true, however, with the fruit of the 1937 crop. Also, the sugars in the fruits from the two plots fertilized with nitrogen alone and with phosphorus and potassium together appeared to be as high or slightly higher than in those fruits receiving other fertilizer combinations. The total sugars showed a larger difference than did the individual sugars. In the second of these two years, however, the fruit from the plot fertilized with nitrogen did not show as high or higher amounts of sucrose and total sugars as did fruit from other plots. Comparing the composition of the fruit from the same plots in two successive years, a variation

was evident, particularly with regard to the amount of dextrose and sucrose. In the second of the two years, the amount of dextrose was somewhat higher. The amount of sucrose was less in the fruit from all of the plots the second year.

It is noted that variations in the different sugars are not always the same from year to year. This coincides with observations made by St. John and Morris (**8**) who pointed out that the level of different carbohydrates in the same variety of fruit might vary considerably in different years. The data presented here should also be interpreted in the light of the natural sample variation earlier pointed out by the same authors. They showed that two samples of apples of the same variety, from the same trees, and picked at comparable stages of maturity, might vary widely in composition.

Acidity and pH.—In table V are also recorded the data for the total acidity of the juice, as measured in terms of milliliters of 0.116 N sodium hydroxide, and also the pH of the juice.

With apples of the 1937 crop the total acidity was slightly lower in fruits from trees receiving applications of nitrogen fertilizers, alone and in combination. With apples of the 1936 crop, however, the total acidity was somewhat lower in fruit from the check plot, while that from the plot receiving nitrogen alone was somewhat higher than in fruit obtained from the other four plots (NPK, NP, NK, and PK).

During 1937 the acidity of all fruit was somewhat lower than that harvested the preceding year. The pH of the juice varied comparatively little although that of fruit from the check and from the PK plots was more alkaline than the others. This was not true the second year when the variation between plots was slight.

Summary

Data showing the mineral and carbohydrate composition of the Jonathan and Winesap apples from a number of plots of trees treated with several different combinations of fertilizer elements are summarized.

Variations in mineral and nitrogen content of the apples are comparatively small, but in a number of instances indicate trends that may be related to variations in terminal growth and in fruit production.

Increases in terminal growth and fruit production oppose increases in percentages of nitrogen, phosphorus, and potassium in the apples as a result of the fertilizers applied.

Since increases in growth and production resulted primarily from added nitrogen, applications of this element, alone or in combination, generally resulted in reduced percentages in the apples of nitrogen, phosphorus, and potassium.

There was some evidence with the 1931 crop of Jonathan apples and the

1933 crop of Winesap apples, that in fruit produced on trees receiving nitrogen applications, the percentage of dry matter was somewhat lower than when the trees received no added nitrogen. This was not true, however, with the Jonathan crop of 1933.

In the apples of the crops of 1931, 1933, and 1937 there was generally a lower percentage of total acidity in fruit from trees receiving nitrogen. This, however, was not true with the 1936 crop. The total acidity varied considerably in the four years that determinations were made. The Winesap apples were somewhat more acid than the Jonathan apples.

Variations in the sugar content of the fruit are somewhat larger in relation to the total amount of sugar present than was found with the minerals. These variations do not appear to show a consistent relation, however, to fertilizer treatments when considered in the light of variations from year to year, and of natural sample variations.

With Jonathan of the 1931 crop and Winesap of the 1933 crop, the percentages of the alcohol-insoluble, acid-hydrolyzable fraction tended to be higher in apples from trees that received no nitrogen fertilizer applications. With the Jonathan of the 1933 crop, however, this was not the case.

In general, the effect of application of the different fertilizer elements on the composition of fruit was relatively small.

It should be pointed out that there was no evidence of a deficiency of either phosphorus or potassium, and that the effects of the fertilizer applications upon the mineral and carbohydrate composition of apples might be more marked if the trees were grown on a soil in which one or both of these elements were deficient, or if much larger amounts involving "luxury" consumption had been applied in the fertilization program.

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PHOSPHORUS ANALYSIS OF PLANT MATERIAL[1]

B. R. BERTRAMSON

Introduction

Although satisfactory methods for the determination of total phosphorus in plant material are available, they are, for the most part, too long and complicated to serve where a great number of comparative tests in studies of phosphorus nutrition of plants are desired. These methods provide for either volumetric or gravimetric determinations. For the former, 2 to 4 gm. of plant material are required; for the latter, 6 to 12 gm. of material are necessary. The amounts of available material are frequently less than these. By means of a colorimetric method used by the author in phosphorus studies, duplicate samples of only 0.25 gm. were found to check quite consistently within the range of 1 or 2 per cent. in routine analyses. This method is similar to the official micro-method in the 1940 edition of "Official and Tentative Methods of Analysis" (**2**) but is somewhat less time consuming.

It is the purpose of this paper to report upon a method for the determination of total phosphorus and the inorganic fraction of phosphorus in plant material.

Total phosphorus

ASHTON (**1**), and WEISSFLOG and MENGDEHL (**14**) proved that ashing of plant material was equally as satisfactory as wet combustion for successful results. ASHTON found that where 2 m.e. of $Mg(NO_3)_2$ were added per gm. of dry plant material, ashing could be carried on at a temperature of 800° C. without loss of phosphorus.

One of the objections to ashing has been the contention that the pyro- and metaphosphates resulting from the high temperatures of ashing are converted with difficulty to orthophosphate. MENGDEHL (**8**) proved, however, that ten minutes of boiling the above salts in N HCl was adequate to convert them to orthophosphate. This was so successful that he devised a method of analysis of pyro- and metaphosphate on this basis.

In an attempt to verify the above findings, 2.5-mg. samples of phosphorus in solution as orthophosphate were placed in evaporating dishes, 2 or 3 m.e. of $Mg(NO_3)_2$ were added, and the solution was evaporated to dryness. Following ignition at 600° to 700° C. for 30 minutes, the ash was dissolved in 10, 15, or 20 ml. of 2 N H_2SO_4, after which 15 ml. of distilled water was added. This was reduced to 5 ml., or less, by evaporation on the steam bath.

[1] Technical Paper no. 389, published with the approval of the Director of the Oregon Agricultural Experiment Station.

The sides of the dish were then washed down and the volume again reduced to 5 ml. on the steam bath. After cooling, the solutions were made up to a definite volume. The results of a series of such tests compared to a standard made up from the same stock solution and in the same manner but omitting the ashing and digesting procedure are summarized in table I. The determinations were made according to the modified TRUOG and MEYER method as described by BERTRAMSON (3), using a KLETT-SUMMERSON photoelectric colorimeter. It was demonstrated in a previous publication (3) that the amount of $MgSO_4$ present in these tests as a result of the subsequent neutralization of the MgO with H_2SO_4 could be 30-fold greater without affecting the test appreciably. From these studies the following procedure was developed and found very satisfactory for routine analysis.

TABLE I

RECOVERY OF PHOSPHORUS FOLLOWING ASHING WITH $Mg(NO_3)_2$ AND BOILING WITH H_2SO_4

SAMPLE NO.	TREATMENT		PHOSPHORUS RECOVERED*	RECOVERY†
	$Mg(NO_3)_2$	H_2SO_4		
	m.e.	*m.e.*	*mg.*	%
1	2	20	2.49	99.6
2	2	30	2.48	99.2
3	3	30	2.50	100.0
4	3	40	2.50	100.0

* The phosphorus ashed in each case was 2.50 mg.
† 2.5 mg. = 100 per cent.

PROCEDURE

Weigh duplicate samples of finely ground and well mixed plant material which has been dried at 60° C. for 48 hours. (The size of sample will be determined largely by the uniformity and amount of material at hand. A 0.5-gm. sample was generally found most convenient where the material had been ground fine enough to pass a 20-mesh screen, or finer, and had been thoroughly mixed. However, 0.1-gm. samples of properly prepared material are adequate.) Place the material in evaporating dishes of approximately 100- to 150-ml. capacity. If the sample used is 0.5 gm., or less, add 5 ml. of 0.5 N $Mg(NO_3)_2$ and 10 to 15 ml. of distilled water. Evaporate to dryness on a steam bath. Remove and allow the dish to dry before placing in the hot muffle furnace. Ignite at 600° to 700° C. until the residue is uniformly gray in color; 30 minutes is usually sufficient time. Remove from the muffle, cover with a watch glass, and allow to cool until liquid can be added without spattering. Then add 15 ml. of 2 N H_2SO_4 and revolve the dish so that the acid comes into contact with all of the ash. Add 15 to

20 ml. of distilled water and place the dish on the steam bath. Evaporate to a volume of less than 5 ml., wash down the sides of the dish with 10 to 15 ml. of distilled water, and again reduce the volume to about 5 ml. (It is doubtful whether the concentration of silica will in any case interfere. If it does, it may be removed at this point by the usual method of dehydration and subsequently taking up the phosphorus in an acid solution.) Remove the dish from the steam bath and add 15 to 25 ml. of distilled water. After the contents have cooled to room temperature, rub down the sides of the dish with a rubber policeman and transfer the contents quantitatively to a 100-ml. volumetric flask, make to volume, and store in a stoppered Erlenmeyer flask until aliquots have been analyzed colorimetrically by the modified TRUOG and MEYER method.

Inorganic phosphorus

It is often desirable to know the amount of inorganic phosphorus in various plant materials. Likewise, if one knows the total phosphorus content of plant material and can determine the inorganic fraction, it is possible to calculate, by difference, the phosphorus fraction that is organically combined. WEISSFLOG and MENGDEHL (**14**) worked out and tested methods for the separation of the total phosphorus of fresh plant material into three fractions—namely, inorganic, soluble organic, and insoluble organic. DE TURK (**5**) made one separation on the basis of solubility in alcohol as well as those mentioned above. EMMERT (**6**) also developed a method for the determination of inorganic phosphorus of fresh plant material, using 1 per cent. by volume H_2SO_4 as the extracting solution. Where fresh material is used for analysis, the processes of obtaining a representative sample and of storing the material for a period of time are complicated. The practice of decolorizing the extracts with charcoal or by other means has been rendered unnecessary by the introduction of the photoelectric colorimeter in which the color values of the extracts may be deducted from the test reading.

It was found that by treating the plant material prepared as for total analysis with a preliminary wetting agent, such as methyl, ethyl, or n-butyl alcohol, the material assumed physical properties very similar to those of fresh plant material and the EMMERT method (**6**) could be applied. Upon investigating the effect of time of extraction and of various treatments during extraction upon the plant material, however, it was found that the 5-minute period of extraction suggested by EMMERT was not sufficient to obtain a constant value. The various methods of treatment are summarized in table II. From these studies it is evident that in the case of finely ground and dried material the triturating or application of the mechanical shaker in extraction is unnecessary. Furthermore, the use of a wetting agent as

mentioned above is superfluous with finely ground and dried tomato plants. (For some materials such a preliminary treatment might be advantageous.) The values obtained after periods of extraction from 30 minutes to 17 hours were found to agree satisfactorily whereas values for less than 30 minutes of extraction decreased slightly as the period of extraction was decreased. The data indicate that extraction with the 1 per cent. sulphuric acid solution as suggested by EMMERT for fresh material is equally applicable to the finely ground and dried material if the time of extraction is extended to 30 minutes.

Satisfactory recovery of phosphorus added to the extracts has been shown, using charcoal to eliminate the extraneous color (**6, 14**). This was checked by the author using the photoelectric colorimeter and omitting the decolorizing treatment. The recovery was found fully satisfactory, indicating that the extraneous organic material did not affect this colorimetric test.

TABLE II

EFFECT OF TIME AND TREATMENTS UPON THE EXTRACTION OF PHOSPHORUS FROM DRIED AND GROUND PLANT MATERIAL

SAMPLE NO.	TREATMENT WITH EXTRACTION	PHOSPHORUS OBTAINED AT VARIOUS TIME INTERVALS OF EXTRACTION					
		5 MIN.	15 MIN.	30 MIN.	60 MIN.	2 HR.	17 HR.
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
68	(1) Wetting agent*; triturated in mortar	1,342	1,450	1,460	1,510	.....	.....
68	(2) In Erlenmeyer; wetting agent; shaken occasionally	1,363	1,442	1,455	1,455	.....	.....
68	(3) Same as (2) except in mechanical shaker	1,400	1,442	1,480	1,480	.....	.....
68	(4) Same as (2) except H_2O as wetting agent	1,442	.....	.....	1,510	.....	.....
88	Same as (1) then placed in stoppered Erlenmeyer	612	.....	.....	.....	673	660
92	Same as for no. 88	506	.....	.....	.....	562	562

* The wetting agent used was absolute methanol.

The stability of organic forms of phosphorus in plant material was demonstrated by MENGDEHL (**8**) when he found that boiling plant material for 15 minutes with N HCl did not bring about appreciable hydrolysis. In order to determine the stability of the filtered extracts, aliquots of several of the extracts from various samples of tomato plants were analyzed for phosphorus immediately following extractions of 5 and 60 minutes and the remainder of the extracts was kept in tightly stoppered Erlenmeyer flasks for periods of 3 to 10 days at room temperature. The results of this study are summarized in table III. The acidity of the extracts was such as to

preclude the activity of microorganisms and increases in inorganic phosphorus of the extracts upon standing for several days could be wholly attributed to hydrolysis of the organic phosphorus; however, only slight increases in inorganic phosphorus were found. This was more noticeable in the 5-minute extractions and indicated that this short period of extraction was of insufficient duration for equilibrium to be established. The stability of the organically combined phosphorus under the above conditions identified it as a definite fraction of the phosphorus contained in plants.

TABLE III

STABILITY OF PHOSPHORUS EXTRACTIONS FROM PLANT MATERIAL

Sample no.	Phosphorus determined in extracts			Percentage increase in P in 3 days	Percentage increase in P in 10 days
	Immediately after 5-min. extraction	3 days after 5-min. extraction	10 days after 5-min. extraction		
	p.p.m.	*p.p.m.*	*p.p.m.*	%	%
16	536	555	.....	3.5	.....
20	714	724	.....	1.4	.....
24	622	642	.....	3.2	.....
28	444	449	.....	1.1	.....
Same time periods as above but with 60-minute extraction					
68	1,490	1,494	1,494	0.3	0.3
96	1,144	1,156	1,174	1.0	2.6

Phosphorus determined in the above manner may be considered as the inorganic fraction, sharply differentiated from the organic fraction. In view of the reproducible results and the foregoing studies regarding treatment and extractions, the following method is proposed for the direct determination of the inorganic phosphorus of plant material and the indirect determination of the organic phosphorus fraction.

Procedure

Weigh out into Erlenmeyer flasks 0.5-gm. samples of finely ground and well mixed plant material which has been dried at 60° C. Add by means of a pipette 50 ml. of a 1 per cent. by volume solution of sulphuric acid. Stopper the flasks and shake vigorously several times during the next 30 minutes. Filter through acid-washed (arsenic-free) filter paper and store the filtrate in stoppered Erlenmeyer flasks. Aliquots of the filtrate may then be analyzed colorimetrically by the modified Truog and Meyer method (**3**).

The modified Truog and Meyer colorimetric method is, briefly, as follows: Pipette an aliquot of the solution, containing 0.002 to 0.07 mg. of phosphorus as orthophosphate into a 50-ml. volumetric flask. Add a drop or two of 0.5 per cent. solution of para-nitrophenol indicator. Bring the volume to approximately 25 ml. with distilled water, add 1 : 1 NH_4OH drop-

wise until a yellow color appears; then back titrate dropwise with 2 N H_2SO_4 until the color just disappears. Add 2 ml. of 2.5 per cent. ammonium molybdate in 10 N H_2SO_4 until the color just disappears. Make up to volume, mix well, and place in a stoppered Erlenmeyer flask. When a series of solutions have been prepared, add to each from a dropper (**3**) 0.23 ml. of 1 per cent. stannous chloride solution of at least 90 per cent. stannous tin. Whether the readings are made by direct comparison or on a photoelectric colorimeter, the tests should be allowed to stand for the same period of time following the addition of stannous chloride as in the case of the standards. The 15- to 20-minute period of standing is recommended.

Effect of ferric iron

The effect of ferric iron upon the color test has been reported by several workers (**4, 11, 12**). Smith *et al.* (**11**) pointed out that 1 p.p.m. of ferric iron in a solution containing 0.2 p.p.m. of phosphorus exerted a negligible

TABLE IV

Effect of ferric iron on the modified Truog and Meyer colorimetric test*

Concentration of P	Percentage error at various concentrations of ferric iron					
	None	2 P.P.M.	4 P.P.M.	8 P.P.M.	16 P.P.M.	32 P.P.M.
p.p.m.	%	%	%	%	%	%
0.1	0.0	3.1	4.2	10.4	.....	.....
0.5	0.0	2.9	.....	.....	.....	.....
1.0	0.0	3.8	5.0	5.5	6.8	47.5

* Tests were made at 23° C. and readings were made 15 to 20 minutes after the addition of $SnCl_2$.

effect but at higher concentrations the errors were appreciable. Their conclusion was that dilution of the solution to the lower limits of the test was the best practical means of avoiding trouble where ferric iron was present. A brief survey of the literature regarding the relative iron and phosphorus content of a wide range of plant material (**9, 13**) indicates that this practice would be applicable in all cases and with but few exceptions the phosphorus content greatly exceeds the iron content of plants thereby eliminating the trouble entirely.

In table IV are summarized the percentage errors resulting from the presence of various amounts of ferric iron at different concentrations of phosphorus. The error with concentrations of ferric iron as high as 4 p.p.m., in the form of ferric chloride, appears to be somewhat similar for high and low concentrations of phosphorus. At 2 p.p.m. the error was approximately 3 per cent. for concentrations of phosphorus from 0.1 to 1.0 p.p.m. Thus, plant material with a ferric iron content 20 times greater than the phosphorus content could be handled by this method with an error of 3 per cent.

plus the experimental error. Such a ratio of iron to phosphorus in the plant doubtless never exists. With a ferric iron content only 2 or 3 fold greater than the phosphorus content, which is found only in unusual instances, the iron would not be a serious handicap.

A simple test of any kind of plant material would quickly indicate whether or not ferric iron is a serious factor. Where agreement to BEER's law holds over the whole range of phosphorus concentrations used in analyzing aliquots, the ferric iron concentration is not interfering with the test.

Discussion

The modified TRUOG and MEYER method is sensitive to less than 0.05 p.p.m. of phosphorus and the foregoing studies verify its accuracy. EMMERT (**6**) pointed out that the weakness of the original method was the instability of the stannous chloride solution. Storage of this reagent under hydrogen in a simple apparatus described by BERTRAMSON (**3**) has eliminated this criticism. The conformity of the test to BEER's law from 0.05 to 1.75 p.p.m. of phosphorus makes the method especially applicable to routine analysis where a photoelectric colorimeter is used. Lower range concentrations may be analyzed satisfactorily by means of a visual colorimeter.

PARKER and FUDGE (**10**) pointed out that the FISKE and SUBBAROW method (**7**) was satisfactory for analysis of solutions containing more than 1 p.p.m. of phosphorus. The modified TRUOG and MEYER method is sensitive to less than 0.05 p.p.m. of phosphorus. Hence, the advantage of the former in being unaffected by 30 p.p.m. of ferric iron is largely nullified by the fact that the same solution could be analyzed by the modified TRUOG and MEYER method simply by dilution to such an extent that the ferric iron concentration would not interfere.

The speed and accuracy of the method make it especially desirable for comparative studies in phosphorus nutrition experiments. Furthermore, by a determination of total and inorganic phosphorus of plant material, the organic phosphorus fraction may be calculated as the difference between the above values. A method whereby the total, inorganic, and organic phosphorus of plant materials may be quickly and accurately determined should be especially useful in working with the complex problems of phosphorus metabolism and interrelated problems in plant nutrition.

Conclusions

1. A method is presented whereby the total and inorganic phosphorus portion of plant material may be determined quickly and accurately.

2. Data are submitted to prove the stability of the organic phosphorus in a one per cent. by volume sulphuric acid solution; thereby demonstrating that it is a definite fraction of the total phosphorus in plant material.

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ROOT MODIFICATIONS INDUCED IN *ZEA MAYS* SEEDLINGS BY IRRADIATING DRY SEEDS WITH SOFT X-RAYS

G. F. Smith and H. Kersten

(WITH NINETEEN FIGURES)

Introduction

This investigation is in part similar to a previous one which described root modifications in *Vicia faba* seedlings grown from x-rayed seeds (**5**). Its purpose is to repeat and supplement these data, using *Zea mays*, so that it might be possible to state more generally the effects of x-rays on plant roots; and also to test the opinion that one effect of x-raying seeds, which appears in the roots during germination, is on factors involved in cavity formation.

In *Zea mays*, cavities of the lysigenous type occur normally in the roots. If the radiation were active in this respect, it might be expected that the process would show variations in rate and extent as compared with controls.

Methods

The radiation referred to as soft x-rays was supplied by an apparatus described in previous papers (**3, 4, 5, 6**). Unsoaked seeds of *Zea mays* were oriented with their embryo sides toward the source of x-ray radiation, 8 cm. from the focal spot of the x-ray tube. The tube was of the gas type, having a copper target. The most intense part of its radiation consisted of the K_α line (1.54 Å). The dose of radiation selected was 30 peak kv., 10 ma. with an exposure time of 100 minutes. This treatment, according to preliminary tests, results in growth cessation during germination. Any exposure time over 30 minutes, under these conditions, also results in this characteristic growth cessation but to a lesser extent. Variations in growth of seedlings germinated from seeds given increasing exposures to x-rays, are given in figure 1.

Following irradiation, the seeds were germinated in moist peat moss at 85° F. The approximate period of germination before growth cessation becomes apparent in treated seedlings is about 8 days. External observations were recorded for this time and histological studies were made of primary roots of 3-, 5-, and 8-day-old seedlings. Fifty roots of each type were examined microscopically, using standard methods of microtechnique.

The characteristic irregularity in arrangement and extent of tissue in seedlings grown from x-rayed seeds, makes it quite impossible to secure histological sections without some distortion. This accounts for the irregularity in some illustrations of the x-rayed material presented throughout the paper.

Results

Figure 2 indicates the general appearance of 8-day-old seedlings grown from x-rayed seeds as compared with normal seedlings of the same age.

The seedlings grown from x-rayed seeds display certain external modifications in their primary roots. These may be briefly listed as: decreased

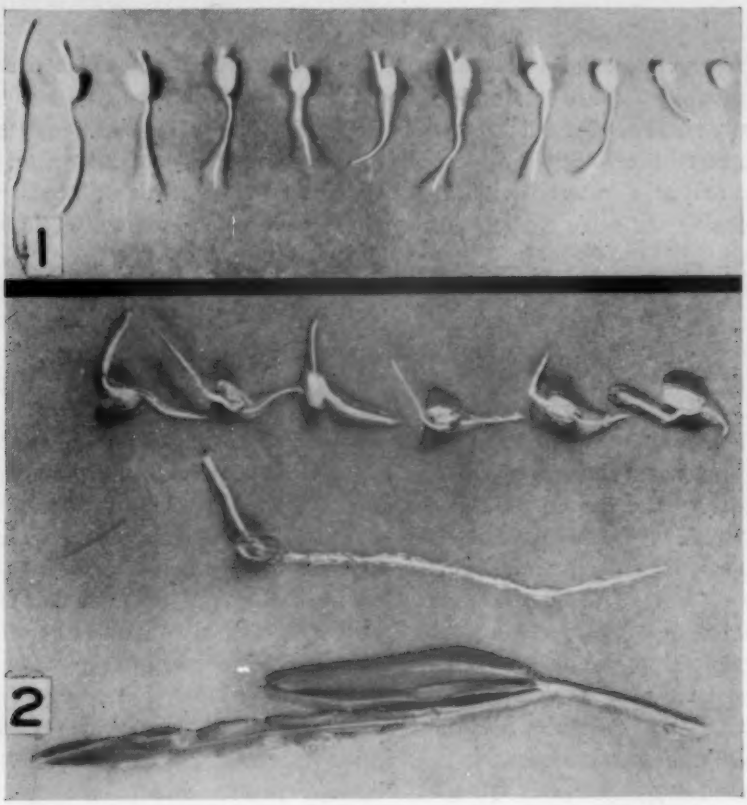


FIG. 1. *Zea mays* seedlings grown from seeds irradiated with soft x-rays for 0, 5, 10, 15, 20, 25, 30, 35, 50, 100 and 200 minutes.

FIG. 2. "Delayed killing" in *Zea mays* seedlings (top) and control plant of the same age (bottom).

root length—an average of 3 cm. in the x-rayed group as compared with an average of 18 cm. in control seedlings; the absence of lateral roots; and a poor development of the root tip. There is also an absence of adventitious roots in the region of the first internode, which are normally formed in *Zea mays*.

A histological study of the primary root tips, examined on the third and

fifth days of germination, shows a relatively normal arrangement of cells of the root tip meristem in seedlings of x-rayed seeds. Root tip sections of 8-day-old seedlings, grown from x-rayed seeds, however, indicate consider-

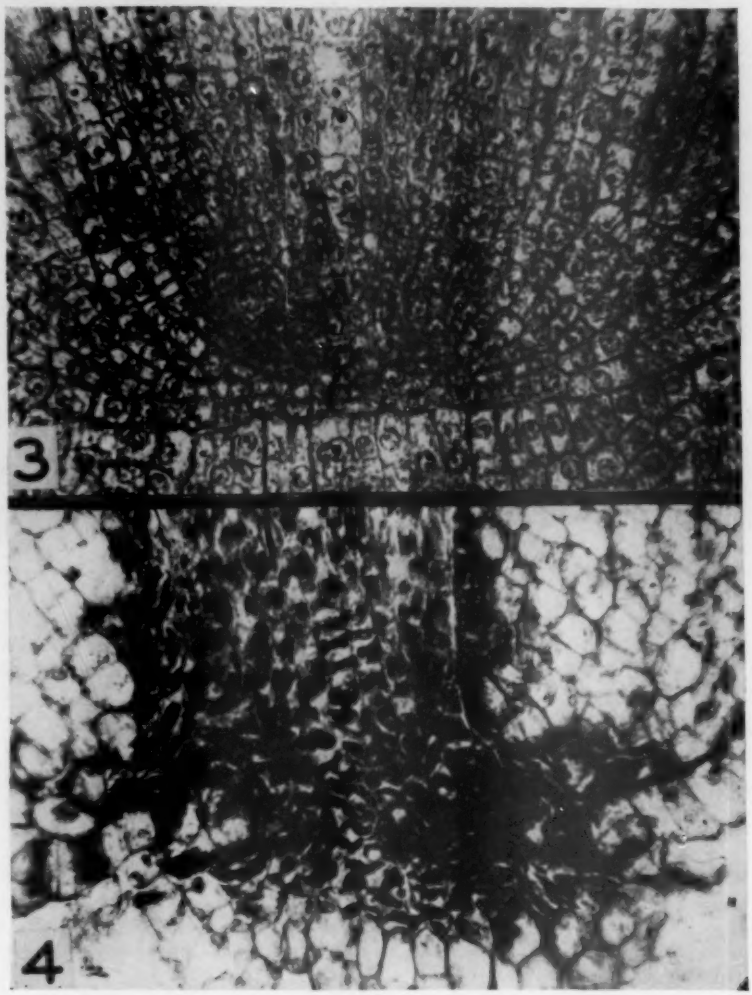


FIG. 3. Normal primary root tip meristem of *Zea mays*. ×250.

FIG. 4. Abnormalities at the root tip meristem in 8-day-old *Zea mays* seedlings grown from x-rayed seeds. ×250.

able distortion and irregularity in size and arrangement of parenchymatous cells. It is also observed that the cellular units of the root-tip meristem are extensively lignified and appear to be in the state of arrested development

(fig. 4). The arrangement of the root tip meristem in normal *Zea mays* seedlings is represented in figure 3.

The extent of activity of the pericycle in lateral root development in 5-day-old corn seedlings of x-rayed seeds is illustrated in figure 6. The state

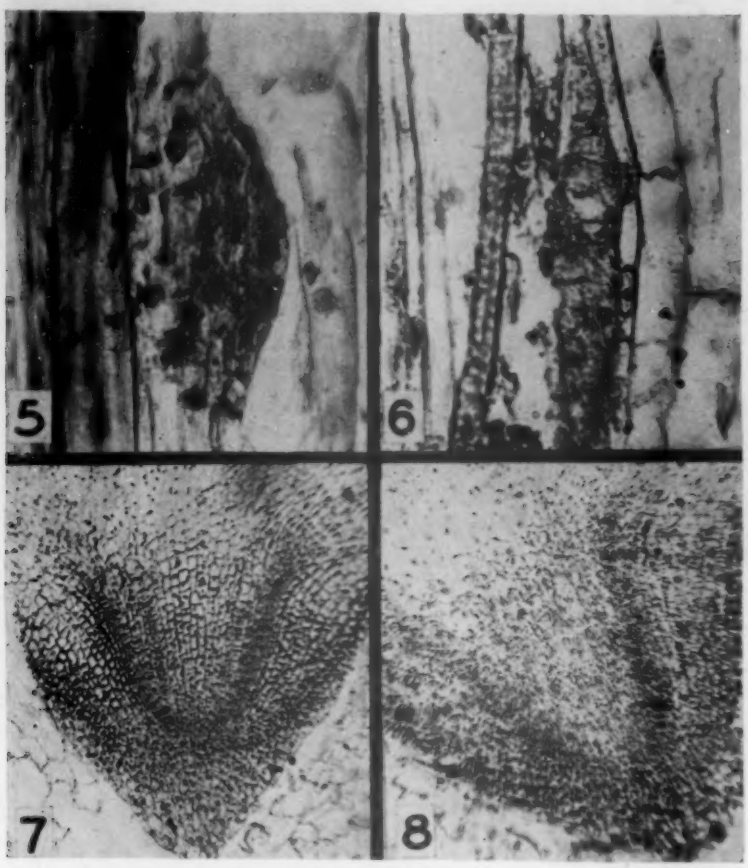


FIG. 5. Normal lateral root initiation in primary roots of *Zea mays* seedlings. ×450.

FIG. 6. Breakdown of pericycle cells in lateral root formation in *Zea mays* seedlings grown from x-rayed seeds. ×250.

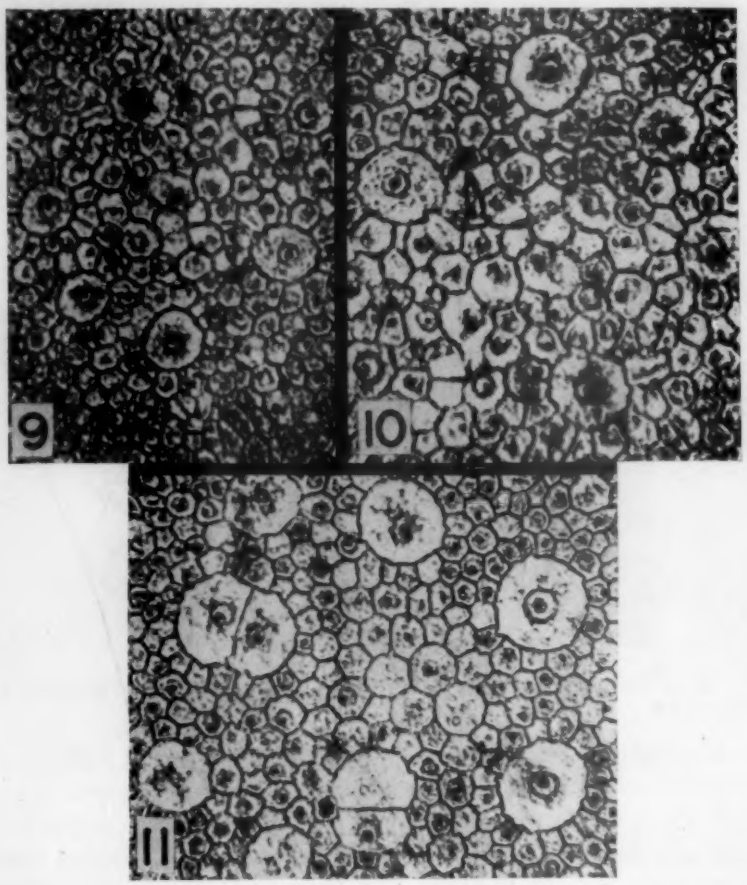
FIG. 7. Adventitious root primordia in the first internode of normal *Zea mays* seedlings. ×125.

FIG. 8. Disintegration of the adventitious root primordia in the first internode of *Zea mays* seedlings germinated from x-rayed seeds. ×125.

of development in control seedlings of the same age is represented in figure 5. In the x-rayed set, lateral root initiation is indicated but the dividing pericycle cells are found to collapse after a few divisions. This accounts for the failure of secondary roots to appear externally.

The failure of adventitious root formation in the internodal regions of *Zea mays* seedlings grown from x-rayed seeds is apparently related to the disintegration of adventitious root primordia. This is shown in figure 8 as it is observed in the first internode of such seedlings. The normal formation of these primordia is represented in figure 7.

An interesting series of observations on the root modifications in *Zea*



FIGS. 9–11. Successive stages in lysigenous cavity formation in the pith region of a normal primary root of *Zea mays*. ×450.

mays seedlings caused by the x-irradiation of seeds involves the formation of lysigenous cavities in the pith region of the primary roots. Normal development of these cavities as found in the control roots are represented in figures 9 to 11. This includes enlargement of specific pith cells which form cavities by the reabsorption of their cellular contents and also enlarge-

ment of certain adjacent cells which divide forming adjoining divisions, which continue to increase in size and later form a single large cavity as the cell walls between them and also their protoplasmic contents are reabsorbed. The end of the process is a more or less definite distribution of 7 or 8 cavities in the pith, each surrounded by regularly arranged pith cells (fig. 12).

In the roots of the 3- and 5-day seedlings grown from the x-rayed seeds, it is found that this cavity formation occurs only as a result of cell enlargement followed by the reabsorption of the protoplasm. No indications of

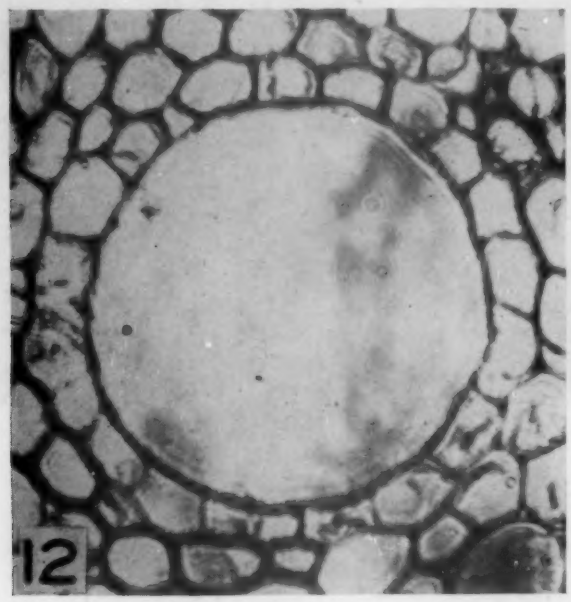


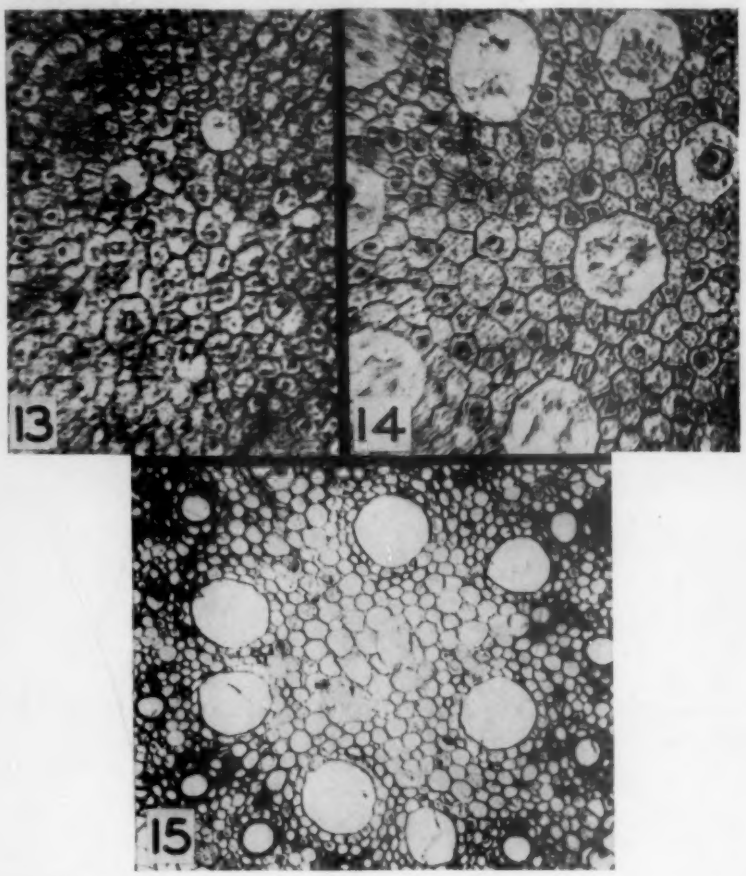
FIG. 12. A lysigenous cavity completely formed in a primary root of normal *Zea mays* seedling. ×675.

cavity formation incorporating dividing cells are observed. These stages of cavity formation in the x-rayed plants are illustrated in figures 13 and 14. A diagram comparing the approximate portion of the root in which these developments occur with those observed in primary roots of control seedlings of the same age is given in figure 19. The modification is very great and may be associated with decreased length.

It is further observed in the 5-day-old seedlings that after the lysigenous cavity formation is complete, to the extent observed in control roots, cell wall splitting occurs in the central portion of the pith (figs. 15 and 16) resulting in schizogenous cavity formation; and also in areas between the originally formed lysigenous cavities (fig. 17), resulting in schizo-lysigenous cavity formations. In the 8-day-old seedlings, these two processes are found

to involve the entire pith portion of the root in its mature region and following this to extend downward to include the pith region of the root tip (fig. 18).

These observations are difficult to interpret. Variations in lysigenous

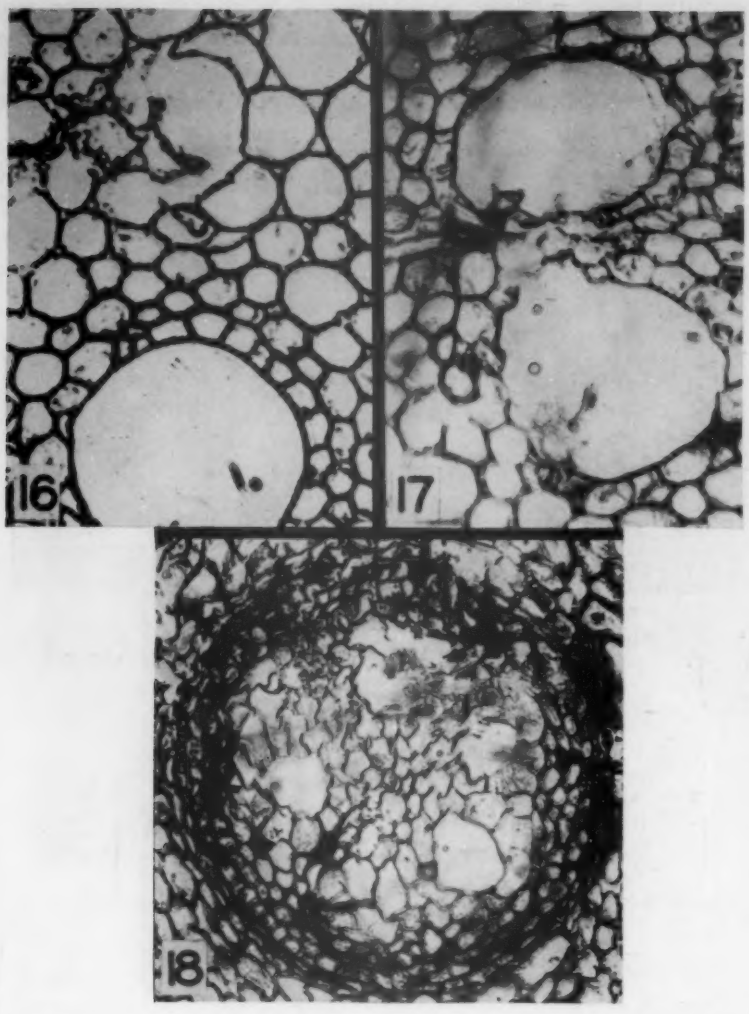


FIGS. 13–15. Lysigenous cavity formation in primary roots of *Zea mays* seedlings grown from x-rayed seeds. Figure 15 shows the beginning of a schizogenous cavity in the pith. ×450.

cavity formation as compared with that in plants grown from non-irradiated seeds, and the additional schizogenous and schizo-lysigenous cavities seem to be two separate effects of the x-ray irradiation.

Summary

External and histological modifications which occur in the primary roots



FIGS. 16, 17. Cell wall splitting of the central pith cells and of cells between lysigenous cavities in primary roots of 8-day-old *Zea mays* seedlings grown from x-rayed seeds. ×675.

FIG. 18. A section showing the extent of cavity formation in the pith region of the primary root tip in 8-day-old *Zea mays* seedlings grown from x-rayed seed. ×450.

of *Zea mays* seedlings grown from x-rayed seeds are described. Microscopical observations of the primary roots of 3-, 5-, and 8-day-old seedlings grown from dry seeds irradiated at 30 kv., 10 ma. for 100 minutes are listed with comparable observations of seedlings grown from non-irradiated seeds.

External root modifications in *Zea mays* caused by the x-ray seed treatment are similar to those found in *Vicia faba:* decreased root length; failure of lateral root formation; and growth cessation after a short period of germination. In addition, adventitious roots do not form in the internodal regions of the seedlings.

Histological observations indicate the following modifications:

1. The primary root tip meristem of the 8-day-old seedlings is extensively distorted and lignified and indicates a condition of arrested growth. The effect, however, is not apparent in treated plants which are from 3 to 5 days old. At this time the meristem cells appear to be relatively normal in arrangement.

2. A breakdown of the pericycle in lateral root initiation is found; also,

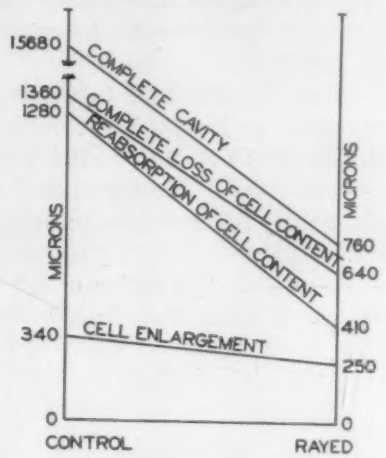


FIG. 19. Diagram indicating the approximate region of lysigenous cavity formation in the primary roots of 5-day-old control seedlings and of seedlings grown from x-rayed seeds. Positions given are measured in micra from the root tip.

a distortion of cells of adventitious root primordia in the first internode is apparent. These correspond to the failure of secondary roots to appear externally.

3. Lysigenous cavities occur normally in the pith region of the primary root of the *Zea mays* used. In the roots of the seedlings grown from the x-rayed seeds, there are slight variations in the formation of these normally present cavities; but a more striking modification is found in their very early and rapid appearance.

4. In addition to these lysigenous cavities in the primary roots, a schizogenous cavity forms in the central region of the pith as a result of cell wall splitting when the seedlings of the x-rayed seeds are approximately from 5 to 8 days old. In the 8-day-old seedlings, schizogenous cavities are observed,

due to continuous cell wall splitting between the previously formed lysigenous cavities and evidences of this cell wall splitting are found to extend downward in the pith region to the lowermost part of the root tip.

5. It is not known whether factors involved in lysigenous and schizogenous cavity formations are comparable. They seem to be separate effects of the x-ray radiation treatment.

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EFFECT OF POTASSIUM IODIDE ON THE ASCORBIC ACID CONTENT AND GROWTH OF TOMATO PLANTS[1]

R. H. Hageman, E. S. Hodge, and J. S. McHargue

(WITH THREE FIGURES)

Introduction

Iodine is one of the common non-metallic elements that is widely distributed in very small quantities in nature. Iodine has a very important function in animal nutrition, which is well established. In plant nutrition the necessity of iodine has not been clearly established. Investigations have been made on the addition of iodine as a fertilizer, the iodine content of various crops, and the effect of iodine on growth and yields of several plants. Mack and Brasher (**5**) give an excellent bibliography of iodine investigations in their publication on the iodine content of certain vegetables.

There is considerable conflict in the reports concerning the response of various crops to iodine treatments. The lack of uniformity of experimental conditions, however, may account for some of these differences. For example, we cite the work of Lewis and Powers (**4**) who found toxic effects of iodides at much lower concentrations than previously reported by Mazé (**6**) until they duplicated his nutrient solution by the addition of chlorides.

The purpose of this investigation was to demonstrate the effect of varied amounts of potassium iodide on the iodine, the mineral, and the ascorbic acid content of tomato plants grown in sand cultures.

Experimental procedure

Early in March, 1941, the seeds of Yellow Pear tomato were sprouted in quartz sand, and four weeks later the seedlings were transplanted into one-gallon earthenware jars. Two plants were placed in each jar which contained 4500 gm. of sand.

The plants were arranged in series and treated as shown in table I. The solution of potassium iodide used for the treatments contained 58.85 grams of potassium iodide per liter. The calculated amount of potassium iodide was added by pouring the required quantity of solution down a glass tube to the bottom of each jar. The addition of the potassium iodide was made in one application ten days after the plants were transplanted. Nutrients were added in solution to each jar from time to time throughout the growing period of the plants. The plants were watered as frequently as necessary to supply their water requirements.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

TABLE I

TREATMENT IN ADDITION TO COMPLETE NUTRIENT SOLUTION*

NUMBER OF JARS	IODINE ADDED ON BASIS OF WEIGHT OF SAND PER JAR
	p.p.m.
7	0 (control)
7	4
7	16
7	36
6	64
6	100

* The nutrient solution was composed of $Ca(NO_3)_2$, KH_2PO_4, $MgSO_4$ and micro amounts of the compounds of twenty-two different elements including a small amount of KI, as described in a previous publication (1).

By observation, the first noticeable injury appeared five days after the addition of the potassium iodide. This injury was characterized by a loss of deep green color, and was progressively followed by the burning of the tips of the leaves on the lower branches, curling and wilting of the leaves, and finally death. The injury advanced upward from branch to branch, and increased progressively in order of the amount of potassium iodide added.

The plants receiving 36, 64, and 100 p.p.m. iodine produced no blooms or

FIG. 1. Plants severely stunted in growth by concentrations of 36 p.p.m. of iodine or more. At all times, the plants receiving 4 p.p.m. of iodine looked as good or better than the control plants. The higher concentrations of potassium iodide seemed to reduce the size of the individual leaves along with general stunting of plant growth.

fruit. The plants receiving 16 p.p.m. iodine produced a few small fruit while those receiving 4 p.p.m. produced as many fruits as did the control plants.

Fourteen weeks after seeding, the plants were harvested by clipping at the surface of the sand. Individual weights of the green plants were taken at the time of harvest. Later, the plants were dried, ground, and analyzed for mineral content. The fruits were also weighed, counted, and then dried, ground and analyzed.

Results

Effect of iodine on the ascorbic acid content of tomato plants

The ascorbic acid content of the plants was determined at intervals of eight, nine, eleven, and thirteen weeks from the time of seeding. Branches were removed from the same relative position on each plant to make the sampling as uniform as possible. Duplicate determinations were made on composite samples from each treatment.

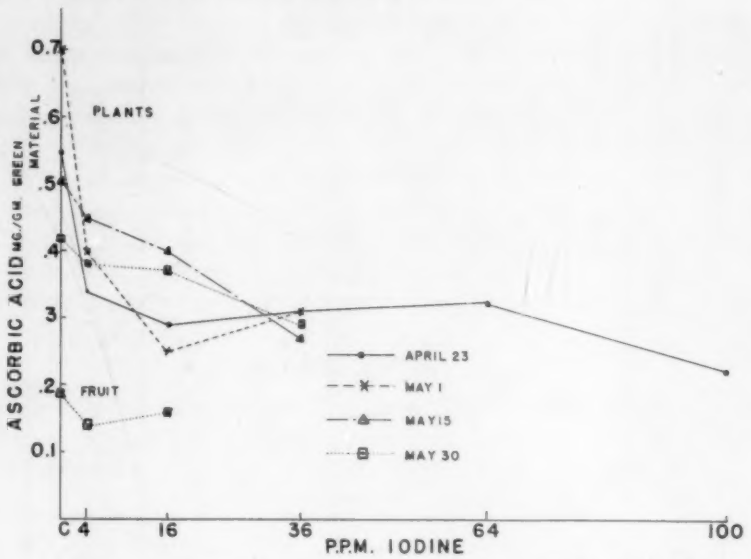

Fig. 2. Ascorbic acid content of tomato plants treated with KI.

The ascorbic acid content of the plants and fruit are given in figure 2. From the data presented it is apparent that as the concentration of the potassium iodide application increased the ascorbic acid content decreased. The effect of the potassium iodide in lowering the ascorbic acid content of the plants was more pronounced before the plants reached maturity. As the plants approached maturity and started to bloom, the ascorbic acid content of the control plants decreased to a level slightly higher than that of the

TABLE II

DRY WEIGHT, MINERAL ANALYSIS AND ASCORBIC ACID ANALYSIS OF TOMATO PLANTS AND FRUITS TREATED WITH INCREASED AMOUNTS OF POTASSIUM IODIDE

TREATMENT	WEIGHT PER PLANT	PERCENTAGE OF CONSTITUENTS (OVEN-DRY BASIS)							PERCENTAGE MOISTURE IN GREEN MATERIAL
		ASH	IODINE	Mn	Cu	Fe	Ca	Mg	
	gm.	%	%	%	%	%	%	%	
PLANTS									
Control	16.2	8.63	0.005	0.0028	0.000075	0.0116	1.64	0.62	83.9
4 p.p.m. iodine	16.2	9.92	0.0470	0.0080	0.000100	0.0146	1.65	0.66	84.0
16 " "	10.8	11.42	0.230	0.0052	0.000125	0.0120	2.02	0.76	82.4
36 " "	4.4	18.28	1.040	0.0080	0.000180	0.0130	2.62	1.12	73.7
64 " "	1.4	21.14	2.500	0.0110	0.000210	0.0110	2.71	1.30	69.4
100 " "	1.0	22.21	3.580	0.0090	0.000222	0.0094	2.72	1.36	53.1

	TOTAL NO. OF FRUIT	AVERAGE WEIGHT	ASH	IODINE	Mn	Cu	Fe	Ca	Mg
FRUIT									
		gm.							
Control	72	5.8	7.17	0.0042	0.0014	0.00020	0.0036	0.18	0.28
4 p.p.m. iodine	76	4.9	7.43	0.0041	0.0010	0.00019	0.0050	0.18	0.38
16 " "	27	2.9	7.67	0.0084	0.0026	.....	0.0087	0.28	0.48

plants treated with 4 and 16 p.p.m. iodine. It was impossible to obtain sufficient uninjured material to determine the ascorbic acid content of the plants treated with 64 and 100 p.p.m. iodine after the initial determination. Although the ascorbic acid content of the fruit grown on the iodine-treated plants was lower than that produced on the control plants, the decrease was not considered to be significant.

Table II shows plant growth and the analyses that were made of both plants and fruits. Plant growth, as measured by the dry weights of the plants, showed increased injury with increased application of potassium iodide. The ash, calcium, magnesium, and iodine content of the plants increased in inverse order of plant growth. The very marked increase of copper and manganese in the plants treated with potassium iodide seems to be of significant importance. It must be noted that the growth, the ash, calcium, iron, and magnesium content were not appreciably altered when the plants were treated with 4 p.p.m. iodine, whereas the increase in the manganese and copper content was very pronounced.

Spectrographic analysis was made of the plants and the values obtained from the plates verified the results obtained for calcium and magnesium content by chemical methods. Spectrographic values also showed a gradual increase in sodium and potassium content of the plants.

Discussion

A possible explanation for the increased uptake of manganese and copper and the rather uniform iron content of the plants may be related to a change in oxidation and reduction equilibrium in the culture.

PIERCE and HAENISCH (**7**) give the following equation for the atmospheric oxidation of the iodide ion.

$$O_2 + 4I^- + 4H^+ = 2I_2 + 2H_2O$$

If we assume that this reaction takes place throughout the sand medium, we then have the following equilibrium.

$$I_2 + 2e = 2I^- \qquad E_o = -0.535$$

It is logical that the iodine would readily react with the existing oxidation and reduction systems. The literature indicates that the oxidation and reduction systems of manganese, copper, or iron seem to be controlled to some extent by the acidity. The acidity of the nutrient solution in all cultures was approximately pH 6.0. Under acid conditions, according to the work of SHERMAN (**9**), the following equilibria are established:

$$Fe^{++} \xrightarrow{H^+} Fe^{+++}$$

$$Mn^{++} \xleftarrow{H^+} Mn^{++++}$$

There are few available data on the cuprous-cupric system. In chemical reactions, however, the oxidation to the cupric form is favored by acid conditions.

According to the work of JOHNSON (**2, 3**) ferric ion is not as readily available to the plant as is the ferrous ion, thus accounting for the reduction in total intake of iron by the plants. It is generally accepted that the divalent manganese is more readily available to the plants than the tetravalent form, thus offering a possible explanation for the increase in the manganese content of the treated plants. According to the work of JOHNSON (**2, 3**), a high level of manganous manganese in soil suppresses the uptake of iron.

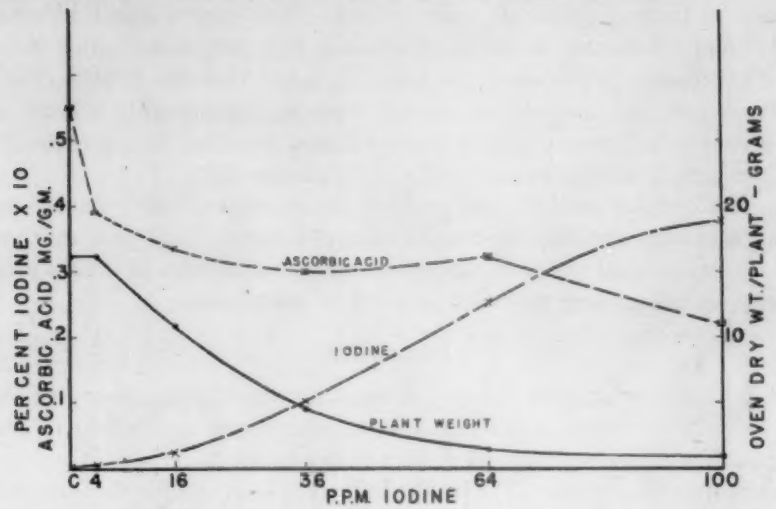

FIG. 3. Relationship of weight, ascorbic acid and iodine content of tomato plants.

In figure 3, the average dry weights of the plants, the average ascorbic acid content of the plants over the growing season and the iodine content of the mature plants are plotted against the iodine treatment. There was no difference between the dry weight of the plants treated with 4 p.p.m. iodine and that of the control plants, although all other applications reduced the growth in order of increased concentration of the potassium iodide.

The percentage of iodine in the plants increased, first gradually and then sharply, as the amount of applied potassium iodide increased. A maximum value of 3.58 per cent. of iodine was found in the plants treated with 100 p.p.m. iodine.

The point of outstanding interest in the experiment was a drop of 30 per cent. in the ascorbic acid content of the plants treated with 4 p.p.m. iodine from that of the control plants. It must be reiterated that by observation and by actual dry weight comparisons there was no difference between the plants

treated with 4 p.p.m. iodine and the control plants, although there was a decrease of nearly one-third in the ascorbic acid content of the treated plants.

WYND (**10**) in his work on the effect of iodine on tomato plants found that: "Respiration, peroxidase, and invertase of the plants were decreased by low concentrations of potassium iodide (1 p.p.m.), but greatly increased at higher concentrations. The increase in catalase at the higher concentrations was much less than that of respiration, peroxidase, or invertase. Oxygenase activity is progressively lowered in the plants growing in higher potassium iodide concentration, thereby differing from the other oxidizing enzymes studied."

WYND's work, which indicated that addition of potassium iodide to the nutrient media seriously disturbed the physiological balance of tomato plants, seems to be in agreement with the marked reduction of ascorbic acid of the plants used in this experiment. From this, also it is to be inferred that the iodine absorbed into the plant probably enters into the oxidation and reduction systems of the plant and seriously interferes with normal metabolism.

The reduction of the ascorbic acid content of the plants treated with 4 p.p.m. iodine, though they exhibited no visible signs of physiological disturbance, indicates the possibility of using ascorbic acid measurements as an index of plant health. SCHMITT (**8**) indicated in his work that changes in ascorbic acid content of the plants reflected unbalanced fertilizer treatments.

At this point, it is desirable to emphasize that this paper presents the data of one series of plants grown in the greenhouse during the spring of 1941. Further studies are now in progress and a duplicate series is planned for the spring of 1942.

Summary

1. A series of tomato plants grown in sand cultures was treated with potassium iodide in concentrations that varied from 4 to 100 p.p.m. iodine.

2. Concentrations of 4 p.p.m. iodine did not reduce the growth of the plants as measured by their dry weights. Applications of 16 p.p.m. iodine or more, seriously injured the growth of the plants and also curtailed production of fruits.

3. Injury was characterized by loss of green color of the plants, necrotic edges of lower leaves, followed by curling, and death. The injury moved progressively upward from branch to branch.

4. Manganese and copper uptake of the treated plants was more pronounced than any of the other mineral constituents.

5. All concentrations of potassium iodide caused a marked decrease in the ascorbic acid content of the plants.

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PRELIMINARY INVESTIGATION OF THE RHYTHMIC FLUCTUATIONS IN TRANSPIRATION UNDER CONSTANT ENVIRONMENTAL CONDITIONS

JUAN C. MONTERMOSO AND A. R. DAVIS

(WITH TWO FIGURES)

Introduction

Rhythmic fluctuations in root pressure were observed by HOFMEISTER (**8**) as early as 1862 and reported on by various others (**7, 13, 14**). Recently this problem was studied more critically by GROSSENBACHER (**6**), who under carefully controlled temperature and humidity conditions obtained additional proof of diurnal changes.

There is a possible relationship of rhythmic fluctuations in root pressure to similar changes in transpiration intensity. LLOYD (**10**) employed the term "induced rhythm" for this latter phenomenon, and KOHL (**9**) described it as "eine von äusseren Einflüssen unabhängige Periodicität." In experiments with Helianthus and Brassica, UNGER (**12**) found a maximum transpiration rate occurred between 12 M. and 2 P.M.; ANDERS (**1**) employing Hydrangea observed the highest rate between 11 A.M. and 3 P.M.; whereas MARCANO (**11**) found that with the tropical plants used (*Colocasia esculenta*, Agave, etc.) the maximum intensity was before noon,—10 A.M. to 12 noon. Because of the lack of exact control these observations are open to question; indeed, BARANETSKY (**2**) questioned the existence of this phenomenon independent of external influences. The recently reported work of BIALE (**3**), however, supports the contentions of UNGER (**12**), LLOYD (**10**), CURTIS (**4**), DARWIN (**5**) and others. BIALE used lemon cuttings and worked under constant environmental conditions.

It has seemed worthwhile to reopen the problem and attempt to obtain evidence under conditions eliminating the temperature and humidity variables. In the experiments herein reported these two factors were carefully controlled. Moreover, the nature of the experimental material—rooted Coleus leaves and cuttings—reduced changes in leaf area due to growth during the experimental period to a minimum. In our experiments this was particularly true of Coleus leaves, the new growth being restricted to roots developing from the petiole. Since the leaves selected had reached maximum size at the time of rooting, a constant transpiration area was obtainable.

Data have been accumulated bearing on the relation of root pressure and concentration of the bathing solution to fluctuations in transpiration; the experiments herein reported, however, are restricted; first, to the reality of rhythmic transpirational fluctuations in rooted Coleus leaves; second, to the

reversal of the diurnal maximum and minimum through the reversal in the light-dark sequence; and third, to the determination of the time required to effect this reversal. Experiment 1 as described, furnished data on the first point, experiment 2 on the second, and experiment 3 on the third. In all these experiments the rooted leaves or cuttings were subjected to a pre-conditioning environment with respect to light-dark periods. The data on transpiration were then collected for experiments 1, 2, and 3 for a period of 75, 72, and 24 hours respectively, on plants in the dark at a constant temperature of 76.5° F. (24.7° C.), and 60 per cent. relative humidity.

Results

Experiment 1

Leaves of *Coleus blumei* which had been rooted in the usual manner under greenhouse conditions were used. Five rooted leaves selected for uniformity were transplanted into 125-ml. wide-mouth Erlenmeyer flasks filled with $\frac{1}{10}$ strength nutrient solution (Hoagland solution).[1]

TABLE I

Average transpiration of five rooted leaves of *Coleus blumei* expressed in grams per dm.^{2} of leaf area per three-hour intervals. Temperature 76.5° F. (24.7° C.). Relative humidity 60 per cent.

Time	Water loss
	gm.
4:30– 7:30 P.M.	0.2372
10:30	0.2084
1:30 A.M.	0.1914
4:30	0.2003
7:30	0.2088
10:30	0.2189
1:30 P.M.	0.1966
4:30	0.1837
7:30	0.1753
10:30	0.1760
1:30 A.M.	0.1865
4:30	0.1920
7:30	0.2062
10:30	0.2059
1:30 P.M.	0.1911
4:30	0.1814
7:30	0.1815
10:30	0.1769
1:30 A.M.	0.1807
4:30	0.1893
7:30	0.1923
10:30	0.1935
1:30 P.M.	0.1906
4:30	0.1889
7:30	0.1893

[1] The composition of the full strength solution on which the dilutions were based was: KNO_3, 0.005 M; $Ca(NO_3)_2$, 0.005 M; $MgSO_4$, 0.002 M; KH_2PO_4, 0.001 M plus 1 ml. of 0.05 per cent. ferric tartrate per liter.

The experimental plants were placed in a dark room at a constant temperature of 76.5° F. (24.7° C.) and relative humidity of 60 per cent. at 1 P.M. February 4, 1941. Loss of water was determined by weighing the plants on a "Chainomatic" balance, the transpiration rate being calculated as grams per square decimeter of leaf area. Leaf area was determined by means of the blue-print method, and a comparison of areas by weight of cut-outs. The first weighing was made at 1:30 P.M. on February 4, 1941. Water loss determinations were made at three-hour intervals thereafter until 7:30 P.M. on February 7, 1941. Table I shows the result of these determinations. While average values only are given and the transpirational rate is characteristic for each leaf used, nevertheless the maximum and minimum for each leaf is identical with the average.

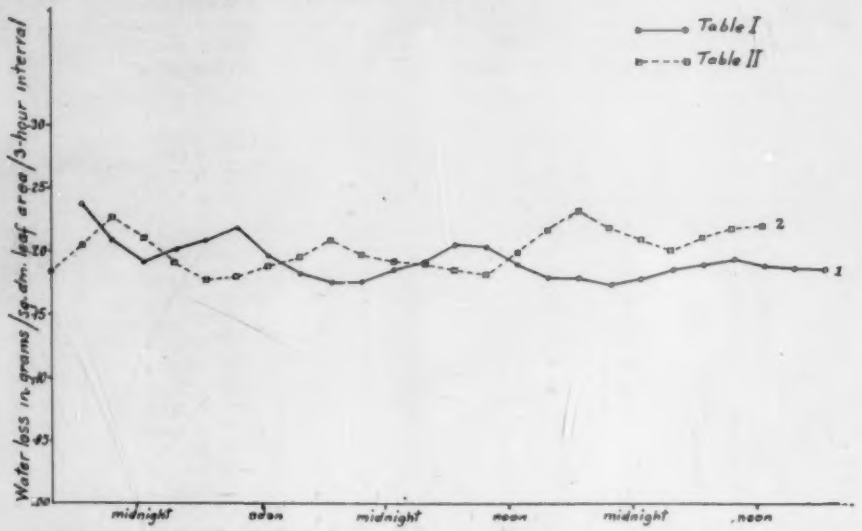

FIG. 1. 1. Transpirational fluctuation in dark after normal light-dark pre-conditioning period. 2. Transpirational fluctuation in dark after reversed light-dark pre-conditioning period.

It will be noted in table I that the periodicity in transpiration is very pronounced, a fluctuation which could not be correlated with any environmental factor during this dark period. Thermograph as well as humidity records showed no diurnal fluctuations.

EXPERIMENT 2

At 7:30 P.M. on February 7, the plants used in experiment 1 were subjected to artificial light of 125 foot-candles light intensity, reversing the light-dark periods for 8 days of 12-hour intervals. Light was turned on at 7:30 P.M. and off at 7:30 A.M.

On February 14 at 7:30 A.M. the same plants were placed again in the

dark. Water loss determinations for each three-hour period and under the above constant conditions were resumed, continuing until 1:30 P.M. on February 17, 1941. Table II shows the results after subjecting the plants to reversed light-dark periods.

This time it will be noted that the diurnal maxima and minima are the reverse of the ones obtained under the conditions of experiment 1. Figure 1 shows graphically the relation between the results in tables I and II. Again,

TABLE II

AVERAGE TRANSPIRATION OF THE SAME ROOTED LEAVES OF *Coleus blumei* USED IN EXPERIMENT 1 AFTER SUBJECTING THEM TO REVERSED LIGHT-DARK PERIODS FOR EIGHT DAYS. TEMPERATURE 76.5° F. (24.7° C.). RELATIVE HUMIDITY 60 PER CENT.

TIME	WATER LOSS
	gm.
1:30– 4:30 P.M.	0.1811
7:30	0.2039
10:30	0.2270
1:30 A.M.	0.2122
4:30	0.1904
7:30	0.1754
10:30	0.1793
1:30 P.M	0.1882
4:30	0.1951
7:30	0.2099
10:30	0.1976
1:30 A.M.	0.1925
4:30	0.1923
7:30	0.1853
10:30	0.1823
1:30 P.M.	0.2008
4:30	0.2195
7:30	0.2349
10:30	0.2211
1:30 A.M.	0.2112
4:30	0.2034
7:30	0.2146
10:30	0.2214
1:30 P.M.	0.2239

Average area of leaves—0.638 $dm.^2$
Average wet weight of roots—0.3372 grams.

it may be said that although the individual plants showed different amounts of water loss, they exhibited maxima and minima for similar time periods, hence the diurnal periodicity of the average became the true reflection of the individual fluctuations. Only the averages are plotted in figure 1.

EXPERIMENT 3

From the preceding experiment it has been found that after subjecting the plants to reversed light and dark periods for 8 days they showed a decided response to light, as shown by the fact that the diurnal maxima and

minima fluctuations are reversed. The purpose of experiment 3 was to determine the time required to effect this reversal.

Rooted cuttings of *Coleus blumei* were employed instead of rooted leaves as in experiment 1. Fifty such cuttings, each with 4 or 5 leaves, were placed in ¼ strength nutrient solution, otherwise under the same conditions as the leaves in experiments 1 and 2. On June 8, 1941, this set of cuttings was illuminated by light from Mazda lamps as follows: at 7:30 A.M. light of 125 foot-candle light intensity was used; at 10:30 A.M. the intensity was increased to 175 foot-candles and at 4:30 P.M. reduced to the original value. Finally, at 7:30 P.M. the cuttings were placed in darkness until 7:30 the next morning. This procedure was repeated for three days before transpiration values were determined. The changing light intensity experiment simulated the intensity changes during a normal day.

TABLE III

TRANSPIRATION OF ROOTED CUTTINGS OF *Coleus blumei* EXPRESSED IN GRAMS PER THREE-HOUR INTERVAL PER PLANT. AVERAGES ONLY ARE HERE RECORDED. TEMPERATURE 76.5° F. (24.7° C.). RELATIVE HUMIDITY 60 PER CENT.

SAMPLES	TIME								
	4:30–7:30 A.M.	10:30 A.M.	1:30 P.M.	4:30 P.M.	7:30 P.M.	10:30 P.M.	1:30 A.M.	4:30 A.M.	7:30 A.M.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1– 5	0.2512	0.2654	0.2545	0.2344	0.2116	0.1995	0.1873	0.2005	0.2113
6–10	.....	0.2204	0.2264	0.2282	0.2523	0.2474	0.2235	0.2037	0.1838
11–15	.....	0.2224	0.2215	0.2253	0.2497	0.2426	0.2304	0.2024	0.1773
16–20	.....	0.2205	0.2077	0.2126	0.2256	0.2334	0.2053	0.1864	0.1753
21–25	.....	0.1685	0.1659	0.1792	0.1957	0.2083	0.1834	0.1556	0.1466
26–30	.....	0.1875	0.1766	0.1935	0.1974	0.2044	0.1765	0.1595	0.1494
31–35	.....	0.1423	0.1334	0.1494	0.1647	0.1727	0.1545	0.1434	0.1346
36–40	.....	0.1664	0.1609	0.1575	0.1865	0.2044	0.1604	0.1425	0.1291
41–45	.....	0.1731	0.1668	0.1720	0.2015	0.2103	0.1712	0.1418	0.1272
46–50	.....	0.1992	0.1854	0.2088	0.2146	0.2302	0.1896	0.1693	0.1601

On June 11, 1941, at 7:30 P.M. samples 1 to 5 inclusive were taken from the above pre-conditioning environment and placed under the constant temperature and humidity conditions governing experiments 1 and 2. Water loss determinations over a 30-hour period and at 3-hour intervals were made on samples 1 to 5 in the same way as in preceding experiments.

The remaining cuttings (6 to 50) were then subjected to reversed light-dark periods, that is, light was on at 7:30 P.M. and out at 7:30 A.M. and from 10:30 P.M. to 4:30 A.M., the intensity employed was 175 foot-candles instead of 125. On June 13, at 7:30 A.M. samples 6 to 10 which had been under reversed light-dark periods for only 1½ days were placed in the dark and weighed as before every 3 hours over a 24-hour period. Each day there-

after 5 samples were placed in the dark and used for water loss determinations. This was continued through cuttings numbered 45 to 50, this lot having been under reversed light-dark periods for 9½ days. Table III shows only the averages of the result of this series of determinations and the graph of the averages in figure 2.

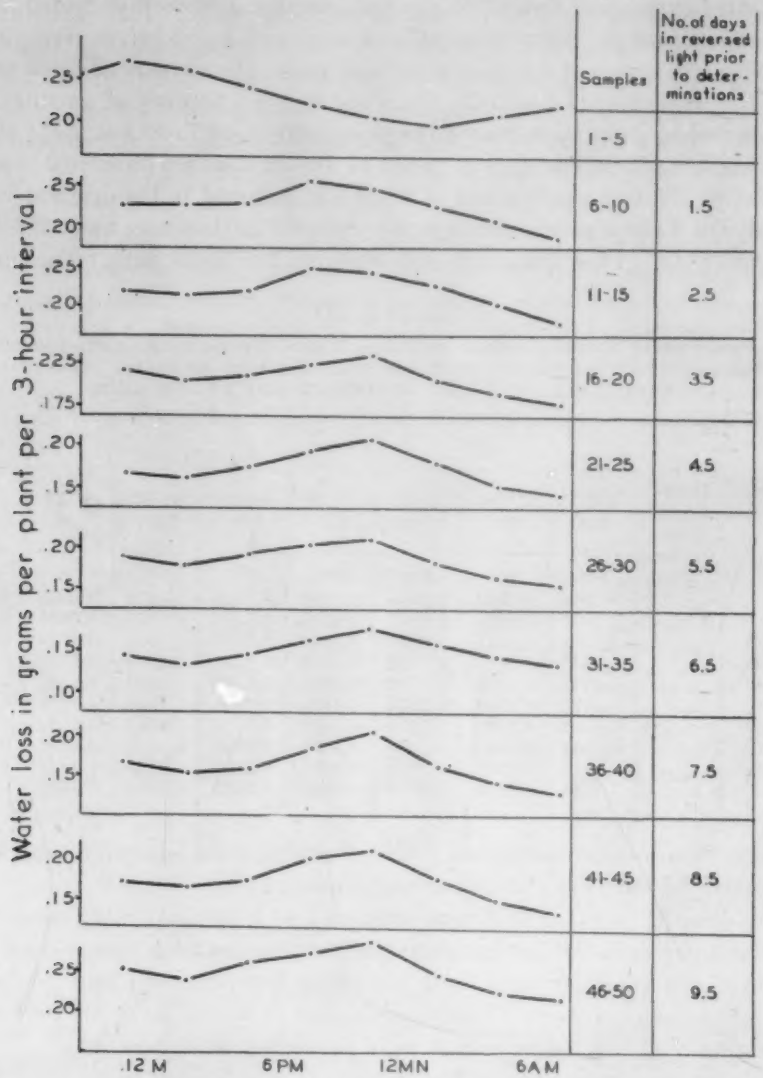

FIG. 2. Gradual transition to complete reversal of maximum and minimum through reversal of light-dark periods.

The results show that samples 1 to 5 (controls) follow the diurnal fluctuations of normal day plants; maximum in the middle part of the day and minimum in the middle part of the night. Samples 6 to 10 which had been under reversed light-dark periods for only 1½ days (2 light and 1 dark periods) early exhibited a decided response. The maximum had been shifted to 7:30 P.M. and minimum to 7:30 A.M. Samples 11 to 15 (2½ days) have the maximum still at 7:30 P.M. but in lots 16 to 20 (3½ days) the maximum has shifted to 10:30 P.M. and the minimum to 1:30 P.M. The maximum and minimum for samples 16 to 50 remained the same as in 16 to 20 (3½ days) but the diurnal maxima and minima fluctuations obtained became more pronounced and presumably more permanent the longer the subjection to the reversed light-dark periods. We noted that there is a gradual shift of the maximum and minimum as shown in samples 6 to 10 (1½ days), and samples 11 to 15 (2½ days) so that in samples 16 to 20 (3½ days) a complete reversal is accomplished.

Further work on this problem of rhythmic fluctuation in transpiration is being continued in this laboratory and the relation of periodicity in transpiration to root pressure and to concentration of the bathing solution will be discussed in the next paper.

Summary

1. Leaves and cuttings of *Coleus blumei* which had been rooted in the usual manner under normal greenhouse conditions showed rhythmic fluctuation in transpiration when placed under constant environmental conditions of darkness, temperature, and humidity.

2. The diurnal maximum and minimum fluctuations could be reversed by subjecting the plants to reversed light-dark periods using artificial light.

3. There is a gradual transition of the maximum and minimum so that within 4 days a complete reversal is accomplished.

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RESPIRATION AND CHEMICAL CHANGES OF THE PAPAYA FRUIT IN RELATION TO TEMPERATURE[1]

WINSTON W. JONES

(WITH TWO FIGURES)

Since the modification of the quarantine laws (**11**) to permit the shipment of fresh fruit from Hawaii to Pacific coast markets and the adaptation of the quarantine treatments to the papaya (**4, 5, 6, 7**) the problem of delivering high-quality fruit to these markets now depends largely on the proper storage temperature. It has been established (**8**) that a temperature below 50° F. "chills" the papaya after which the fruit will not ripen normally. There has been very little work on the nature of this chill effect, work on the cold storage of the papaya having been confined largely to a study of the proper temperature to apply during transportation. STAMBAUGH (**9**) found that unripe papayas would not ripen after any appreciable time below a temperature of 60° F., while JONES and KUBOTA (**8**) showed that a temperature of 32° to 34° F. for a period of 11 days prevents ripening and that the fruits quickly decay at room temperature. This decay is largely initiated by a latent infection of anthracnose (**7**). WARDLAW *et al.* (**12**) pointed out that the natural resistance of the fruit to the development of latent infection is destroyed by "chill" and by certain chemical treatments. The present paper deals with the nature of the "chill effect" as revealed by the respiratory activity and chemical changes at various temperatures.

Materials and methods

The fruits used in this study were all of the Solo variety and were harvested firm-ripe as needed. Each treatment was run in duplicate at least twice and in some cases four times using 12 fruits for each treatment. The storage temperatures used were 40°, 45°, 50°, 55°, and 60° F. (maintained ± 1° F.) for the 5-day storage period. The 5-day storage period was selected as being equivalent to the time required for the fruit to reach Pacific coast markets from Honolulu. The fruits used for chemical studies were treated as follows: (1) Analyzed immediately after harvest; (2) allowed to ripen at room temperature; (3) held 5 days at a specified temperature; and (4) held 5 days in cold storage and then allowed to ripen at room temperature or until the fruit from the low temperature first showed signs of decay.

Methods of analysis were standard. Sugars were determined by the method of STILES, PETERSON, and FRED (**10**). Soluble pectin was determined

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by precipitation with $CaCl_2$ of a cold water extract (7 extractions) of the finely ground residue from an alcoholic extraction. Carbon dioxide was found by absorption in LiOH and, after precipitation of the bi-carbonates and carbonates with $BaCl_2$, titration of the excess alkali with 0.1 N HCl to phenolphthalein.

Results and discussion

Respiration in relation to temperature

Work on the storage of the papaya has shown that the critical temperature lies slightly below 50° F. This is shown more clearly in figures 1 and 2.

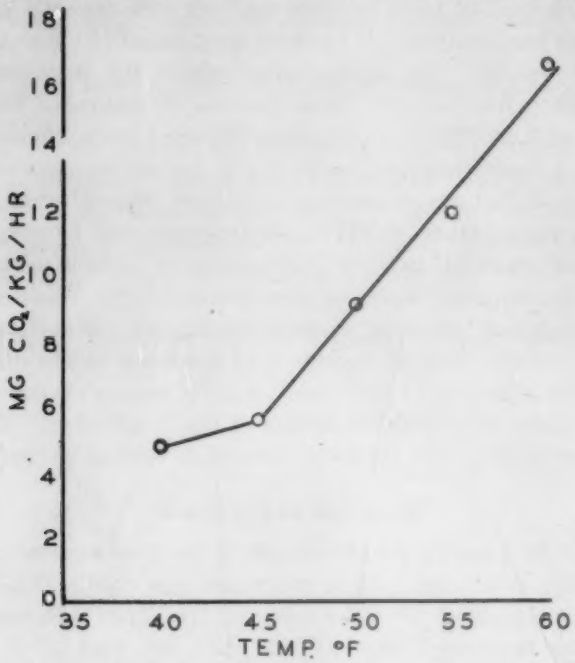

Fig. 1. The influence of temperature on carbon dioxide production of papaya fruit.

The production of carbon dioxide in milligrams per kilogram of fruit at the different temperatures was as follows: 40° F. – 4.8; 45° F. – 5.5; 50° F. – 9.0; 55° F. – 12.0; 60° F. – 16.0. The temperature coefficient over the same temperature range was 1.5 between 40° and 45° F., 3.3 between 45° and 50° F., 2.2 between 50° and 55° F., and 2.2 between 55° and 60° F. This does not agree with the findings by Haller *et al.* (2) for strawberries, peaches, lemons, oranges, and grapefruit in which they report a higher Q_{10} for the lower temperature range. Thus there is a marked break in the nature of the processes having to do with the production of carbon dioxide in the papaya between the temperatures of 45° and 50° F. The critical

nature of the temperature between these two points is further shown in the chemical changes as will be discussed later, and in the ripening of the fruit after a period at this temperature. On removal to room temperature after 5 days at 45° F. the papaya fruits ripen unevenly, with poor quality, and are subject to the development of anthracnose, while after a like storage period at 50° F. the fruits ripen evenly, with high quality and no decay.

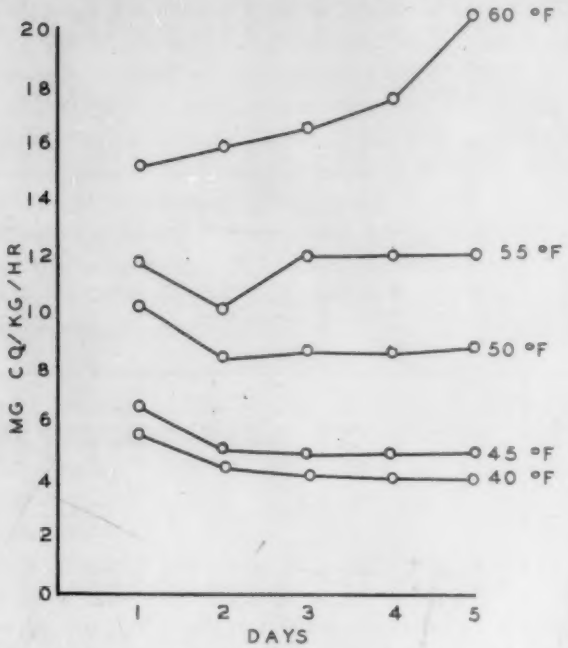

FIG. 2. Carbon dioxide production of papaya fruit for a period of five days at the temperatures indicated.

CHANGES IN CHEMICAL COMPOSITION IN RELATION TO TEMPERATURE

It has been shown (**8**) that the most marked changes in chemical composition during ripening of the papaya are the reducing sugar-sucrose relations, a reduction in structural materials (acid hydrolyzable materials), and an increase in soluble solids. Further, a temperature of 32° to 34° F. stops these changes and they are not resumed on removal to room temperature. Table I shows the chemical changes occurring during storage over the temperature range of 40 to 60° F. and during subsequent ripening.

An examination of table I shows that there is very little hydrolysis of sucrose during storage at the lower temperatures and on removal to room temperature the hydrolysis of the sucrose does not proceed as far in the chilled fruit as in the check fruit. At the higher temperatures (50° F. and above) there is a slow hydrolysis of sucrose. This hydrolysis is continued

TABLE I

CHANGES IN COMPOSITION OF THE PAPAYA FRUIT DURING STORAGE AND SUBSEQUENT RIPENING. PERCENTAGE OF FRESH WEIGHT

STORAGE TEMPERATURE	FRUIT TREATMENT	SOLUBLE SOLIDS	INSOLUBLE SOLIDS	REDUCING SUGAR	SUCROSE	TOTAL SUGAR	SOLUBLE PECTIN*	ACID HYDROLYZABLE
°F.		%	%	%	%	%	%	%
	Analyzed immediately	10.01	2.97	4.77	2.95	7.72	0.49	0.64
	Ripened at room temperature	10.22	2.52	7.21	0.76	7.97	0.58	0.52
40°	5 days at 40° F.	10.05	2.76	5.71	2.20	7.91	0.47	0.67
	5 days at 40° F. ripened at room temperature	10.82	3.09	7.52	1.46	8.98	0.65	0.65
	Analyzed immediately	11.34	2.82	6.16	3.40	9.56	0.46	0.72
	Ripened at room temperature	11.62	2.89	8.54	1.09	9.63	0.65	0.63
45°	5 days at 45° F.	12.08	3.00	5.46	4.65	10.11	0.49	0.82
	5 days at 45° F. ripened at room temperature	12.15	2.87	7.84	1.92	9.76	0.62	0.69
	Analyzed immediately	9.25	2.41	4.88	2.34	7.22	0.35	0.66
	Ripened at room temperature	9.65	2.77	6.98	0.68	7.66	0.67	0.66
50°	5 days at 50° F.	9.74	2.34	5.96	1.92	7.88	0.48	0.60
	5 days at 50° F. ripened at room temperature	10.04	2.87	7.25	0.59	7.84	0.67	0.66
	Analyzed immediately	9.63	2.97	4.34	3.46	7.80	0.39	0.75
	Ripened at room temperature	10.98	2.92	7.71	1.09	8.80	0.65	0.59
55°	5 days at 55° F.	9.61	2.94	4.61	3.11	7.72	0.40	0.83
	5 days at 55° F. ripened at room temperature	10.46	3.36	6.78	1.43	8.21	0.77	0.76
	Analyzed immediately	9.43	3.66	4.21	2.92	7.13	0.53	1.08
	Ripened at room temperature	10.50	3.19	6.47	1.90	8.37	0.71	0.77
60°	5 days at 60° F.	9.57	3.23	5.32	2.65	7.97	0.52	0.87
	5 days at 60° F. ripened at room temperature	10.63	2.91	6.79	2.09	8.88	0.59	0.60

* As calcium pectate.

to completion on removal to room temperature as compared to check fruits. Even though the temperature effect is obvious on the sucrose changes, none of the chemical changes is so markedly affected as is respiration.

This indicates that the basic metabolism of the fruit has been upset rather than simple enzymatic changes. The nature of the basic upset is not clear but on the basis of a temperature coefficient of 1.5 between 40° and 45° F. it is probable that some physical phase of respiration has become limiting. Tropical fruits such as banana and papaya (**3, 8, 13**) are subject to this temperature "chill" at temperatures below 50° F. as contrasted to temperature climate fruits, such as the apple (**1**), which are not "chilled" by temperatures even as low as 32° F. Does this signify a fundamental difference in the basic metabolism of tropical and temperate climate fruits? This question should be carefully investigated.

The results presented show clearly that in the transport and marketing of the papaya the temperature should not be allowed to drop below 50° F. Thus special care will be required in handling papayas on northern markets during the winter months.

Summary

1. Papaya fruit are "chilled" when stored for a period of 5 days at a temperature of 45° F. or lower.

2. The temperature coefficient was found to be 1.5 between 40° and 45° F., 3.3 between 45° and 50° F., 2.2 between 50° and 55° F., and 2.2 between 55° and 60° F.

3. Ripening changes, especially sucrose changes, are retarded by all temperatures studied.

4. It is suggested that there is a difference between the basic metabolism of tropical climate and temperate climate fruit.

5. In the transport and marketing of papayas the temperature should not be allowed to go below 50° F.

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EFFECT OF DROUGHT ON THE PRODUCTION OF PLANT PIGMENTS

WILLIAM A. BECK

It was established previously that light and heat influence pigment production in plants more than any other environmental factors, and that drought plays only a minor rôle in the cause of the seasonal variation of the pigment present in field plants. The conclusion regarding the influence of soil moisture was arrived at from direct experimental evidence and data furnished by the Abbe Meteorological Observatory in Cincinnati (**3**). The work of FÜRLINGER and the conclusions drawn from it caused reasonable doubt regarding the minor importance of soil moisture. FÜRLINGER (**4**) proved that excised sunflower cotyledons fail to become green when they are plasmolyzed in sugar solution and are exposed to light. If the same cotyledons are deplasmolyzed, however, they become green when they remain exposed to the light. The conclusion is drawn that plasmolysis influences the physiological activity of the protoplasm in such a manner that the production of chlorophyll is inhibited. It is the purpose of the present work to study the influence of drought on pigment production more carefully and to determine quantitatively the amount of chlorophyll, xanthophyll, and carotene produced under varying drought conditions.

Methods

In some of the experiments the soil was permitted to dry naturally but in most of them an artificial or "physiological" drought (SCHIMPER's expression) was induced by saturating the soil with sugar solution. In the control experiments the soil was saturated with water. The greatest concentration of the sugar solution employed was 0.38 mol. The osmotic pressure of this solution is 10.6 atm. (**6**) which is equivalent to the maximum suction tension found in the hypocotyl of the seedlings employed. The maximum suction tension of the hypocotyl was found in the cortical tissue directly beneath the cotyledons. Sugar solution of this concentration in the soil effectively prevents any water from the soil reaching the cotyledons. Graded sugar solutions of lesser concentrations were also employed. In other experiments the cotyledons were excised and submerged in water or hypertonic sugar solution during the period of irradiation. The amount of pigment present in the seedlings which were not irradiated and not treated was also determined so that the effect of the various factors might be estimated on the basis of the initial amount of pigment present. Sunflower seeds of the same strain and harvest were employed in order to reduce, as much as possible, the individual differences of the test plants. The seeds were planted in pots filled with

light garden soil. The seedlings were 120 hours old when the tests were begun. They were raised in complete darkness in an incubator at 25° C. and 90 per cent. relative humidity. The etiolated plants were exposed to diffuse daylight in the garden where light from the north sky struck them directly. The experiments were carried out on sunny days during the month of July, the tests lasting 7 hours (from 8:00 A.M. to 3:00 P.M.). No direct sunlight ever struck them. The temperature was approximately the same for the different days. Each day control experiments, in which the soil was flooded with water, were made so that any influence of factors, other than soil moisture on different days, might be detected by comparing the results of the control experiments of different days. The pigments were extracted according to the directions given by SCHERTZ (**5**). The quantitative determination of the amount of pigment present at the end of the experiment was made according to the method previously described (**2**). The averages of the results of the various series of experiments were assembled and recorded in table I.

TABLE I

AMOUNT OF PIGMENT DEVELOPING IN ETIOLATED SUNFLOWER SEEDLINGS UNDER VARYING DROUGHT CONDITIONS WHEN IRRADIATED FOR SEVEN HOURS IN DIFFUSE DAYLIGHT

CONDITIONS	SERIES	AMOUNT OF PIGMENT: MILLIGRAMS PER 1,000 COTYLEDONS		
		CHLOROPHYLL	XANTHOPHYLL	CAROTENE
		mg.	*mg.*	*mg.*
Cotyledons excised:				
Cotyledons immersed in water	1	11.7	9.00	2.00
Immersed in 1-mol sugar solution	2	0.0	10.60	1.06
Plants intact:				
Not irradiated; soil not treated	3	0.0	4.46	0.39
Irradiated; soil not treated (drying)	4	32.5	8.80	1.80
Soil saturated with sugar solution:				
0.0 mol (pure water)	5	24.3	8.10	1.39
0.095 mol	6	24.8	8.00	1.50
0.190 mol	7	38.5	10.80	2.80
0.285 mol	8	44.0	5.70	1.50
0.380 mol	9	22.3	6.70	1.20

Results and discussion

When sufficiently concentrated solutions were applied to the soil, drought manifested itself in the hypocotyl by wilting, beginning at the base and extending upward by degrees. When the maximum concentration was employed, even the cotyledons finally became wilted. This fact must not be interpreted to mean that the sugar solution actively withdrew water from

the cotyledons. Late in the experiment water could no longer be supplied to the cotyledons to cover the loss by transpiration, and in consequence the cotyledons had to become wilty. The reduction of water supply to the cotyledons never occurred suddenly. When the soil was saturated with 0.19 mol solution, wilting manifested itself very slowly, and the support which was necessary for wilting plants was supplied very late in the experiment. No support was required when a solution of 0.095 mol was employed.

When the cotyledons were submerged in 1 mol sugar solution (table I, series 2) no chlorophyll developed but some xanthophyll and carotene developed; this is made evident by comparing the amount of pigment present at the end of the experiment with the amount initially present (table I, series 3). These results are in agreement with those obtained by FÜRLINGER who did not measure the different amounts of pigment present but was satisfied to note qualitatively if chlorophyll developed or not. The development of carotenoid pigment, in spite of plasmolysis, is interesting and perhaps significant. When the cotyledons were submerged in water (table I, series 1) chlorophyll and carotenoid pigments developed. Relatively little chlorophyll, but considerable amounts of carotenoid pigments, developed. The amount of chlorophyll is considerably less than that developed under severe drought conditions (compare the results in series 9). Flooding the tissues evidently depresses the development of chlorophyll. This depression might be caused by the lack of oxygen supply and lack of carbon dioxide elimination. The water-logged condition of the active tissues did not affect the production of carotenoid pigment in the same way; in fact, more was present under this condition than when extreme drought prevailed. If the lack of oxygen supply adequately explains the reduction of chlorophyll production then considerable production of carotenoid pigment suggests that their formation depends less on the respiratory mechanism of the protoplasm than does the production of chlorophyll. This is in agreement with a previous conclusion that carotene and xanthophyll probably form a respiratory mechanism for the protoplasm (**1**). This interpretation would explain why some chlorophyll develops in spite of unfavorable respiratory conditions since the carotenoids might conceivably aid respiration. As was shown in previous experiments (**1, 3**), and is again evident from the present results, the external factors of the environment do not influence the production of chlorophyll and the carotenoid pigments in the same manner; or if they do, not in the same degree.

Comparing the results obtained in the series of experiments from 5 to 9, it becomes evident that water saturation of the soil is not a favorable factor for pigment development. When the soil is saturated with water not much more pigment develops than when extreme drought prevails (compare series 5 and 9). The optimum soil-moisture condition is not the same for chloro-

phyll and carotenoid pigment development. For chlorophyll development it is somewhere between 0.19 mol (equivalent to 5.1 atm. resistance to water uptake) and 0.38 (equivalent to 10.6 atm.). It is approximately 0.285 mol [(equivalent to 7.75 atm.) (series 8)]. For the carotenoid pigments the optimum is approximately 0.19 mol [(equivalent to 5.1 atm.) (series 7)]. No effort was made to estimate the actual resistance which the soil offers to the water uptake by the seedling, but the osmotic pressure of the solution which flooded the soil was taken as a satisfactory indicator of the resistance offered. It is interesting, and probably significant, that the maxima occurred for the two carotenoid pigments before that of the chlorophyll, just as the maxima for the carotenoids in seasonal variations occur before those of the chlorophyll (**3**). In view of the results of present and previous work, it is easy to understand that in this region the least amount of pigment is present in field plants during the months of January and February when heat, light, and drought offer the most unfavorable conditions for pigment production.

Conclusions

The following conclusions may be drawn from the results of the present experiments:

1. Plasmolysis of the active cells inhibits the production of chlorophyll but does not prevent the development of xanthophyll and carotene.

2. Flooding the active tissue does not prevent the production of chlorophyll but does not favor it. Flooding does not greatly depress the production of xanthophyll and carotene.

3. Saturation of the soil with water does not favor the production of plant pigments. It affects the chlorophyll production more than it does the production of carotenoid pigments.

4. There are optimum soil moisture conditions which are not the same for the three pigments. Expressed approximately in atmospheres in function of the osmotic pressure of the sugar solutions with which the soil was flooded, the optimum for chlorophyll is 7.75 atmospheres, and for the carotenoid pigments 5.1 atmospheres.

5. Only extreme drought seriously affects pigment production appreciably. The production of carotenoid pigment is affected more readily by it than is that of the chlorophyll.

6. The conclusion previously arrived at, that heat and light are the major factors which influence the production of plant pigments and that drought plays only a minor rôle, is confirmed by the present work.

Summary

The influence of drought on the production of the plant pigments chlorophyll, xanthophyll, and carotene was determined. Sunflower seedlings, in

pots filled with garden soil, served as test specimens. The soil was allowed to dry naturally and artificial drought was induced by saturating the soil with sugar solutions of concentrations ranging from 0.0 to 0.38 mol. In some cases excised cotyledons were immersed in water or sugar solution. The etiolated plants were irradiated with diffused daylight. Heat and light conditions were about the same in all of the experiments. Plasmolysis prevented the development of chlorophyll but not the development of the carotenoids. Water saturation of the active tissue and of the soil is an unfavorable condition. Only extreme drought seriously affects the production of pigments. The optimum soil-moisture condition is different for chlorophyll and the carotenoids. For chlorophyll an equivalent resistance to water uptake of 7.75, and for the carotenoids of 5.1, atmospheres may be taken as fair estimates of optimal conditions. The previous conclusion that heat and light are the major factors which influence pigment production and that drought plays a minor rôle, was confirmed.

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A GRATING SPECTROGRAPH FOR MINERAL NUTRITION STUDIES[1]

R. C. NELSON[2]

(WITH TWO FIGURES)

Introduction

The advantages of the spectrograph as a means of performing qualitative and quantitative analyses for metals and for certain non-metallic elements are well recognized. The method is rapid, accurate and economical and may be applied to very small samples. Furthermore, the spectrographic plate gives a permanent record of the quantities of all the constituents in a sample, although only one or two may have been of interest at the time the analysis was performed.

The spectrograph has not been much used in plant physiological research largely because the initial cost of instruments suitable for quantitative analysis of plant materials is rather high. The present paper describes a relatively inexpensive spectrograph which has been used with considerable success for the analysis of plant ash.

Description of apparatus

The instrument utilizes a concave diffraction grating replica as a means of dispersing light. These grating replicas are available commercially at low prices and in excellent quality; they have the property of dispersing light and bringing the spectrum into focus without the aid of lenses. More specifically, if the grating and a slit be placed upon the circumference of a circle of diameter equal to the radius of curvature of the grating, then the spectra produced will be brought into focus upon the circumference of that circle. A description of the properties of concave gratings may be found in any textbook of optics.

The disposition of parts of the apparatus is shown in figure 1. A is the source of light which is to be dispersed, and B is a quartz sphero-convex lens used to give a uniform distribution of light on the slit C. Light passing through the slit falls on the grating D, where it is dispersed and a spectrum focused upon a photographic film at EF. The grating and film are enclosed in a box which light may enter only through the slit.

In this instrument, the parts are so arranged that the angle CDG is 21°, G being the midpoint of the photographic film. Under these conditions,

[1] Paper no. 1955 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

[2] American Potash Institute Fellow.

using a grating having 25,000 lines to the inch and a focal length of 106 cm., the spectrum from 2450 Å to 4650 Å is brought into focus on a surface nine inches long. This range includes suitable lines for the quantitative analysis of all the elements commonly occurring in plant ash which may be determined with the spectrograph.

The dispersion obtained here, 9.6 Å per mm., is about the minimum dispersion suitable for work with plant ashes, since a smaller value would not permit the clear separation of the boron doublet at 2496.8 Å–2497.7 Å in the first order and might even interfere with the evaluation of the density of the lines of the magnesium quintet. This interference would make the use of a 15,000-line grating undesirable, although the author is informed

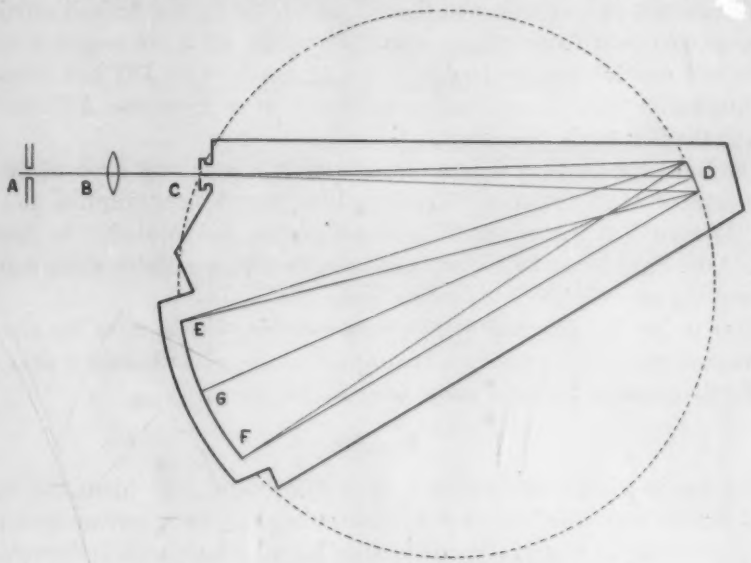

FIG. 1. Diagram of concave grating spectrograph.

that 15,000-line replicas as a class are of better quality than 25,000-line replicas.[3]

If a 15,000-line grating be substituted for the 25,000-line grating in the instrument here described, and the grating be rotated about its vertical axis through a small angle, the visible and near ultra-violet regions are thrown upon the film and may be used for photographing absorption spectra.

Roll film is used to record the spectrum. Cut film might also be used, but because of the short focal length of the grating, plates are not satisfactory. Roll film has the advantages of being cheap and readily obtained, and

[3] Private communication from Dr. M. N. STATES of the Central Scientific Company. It was also stated that the author's 25,000-line replica was of unusually good quality and that equal results could not be expected from all such replicas.

of permitting daylight loading; it holds the focal curve sufficiently well to maintain sharp focus over the entire surface. Kodak Verichrome V–620 film is used. The Eastman Kodak Company Research Laboratory states[4] that in the region specified this film is suitable for quantitative spectrography. Three series of spectra nine inches long may be recorded on a roll, and using a slit 5 mm. high, eight exposures may be made in each series. The film is developed for 105 seconds in D–72 (1:1) on an ordinary development tank reel, using continuous agitation.

After certain requirements are met, the details of construction are dictated chiefly by considerations of convenience, and will not be specified here. The grating must be mounted, however, so that it may be adjusted to bring its rulings into parallelism with the slit, and to bring the desired portion of the spectrum into focus on the film. For this, all three angular adjustments and one longitudinal adjustment along the axis DG are necessary. The filmholder must be adjustable vertically so as to expose different portions of the film to the spectrum.

The light source used is a carbon arc with a potential drop of 50 volts DC, operated at 25 amperes. The sample is placed on a graphite electrode and "burned" in the arc until it is completely evaporated. In order to exclude the light from the hot carbons from the slit, a suitable mask is placed between the arc and the condensing lens.

Except for the slit and the grating replica, which were bought, this instrument was constructed in a laboratory equipped with only a drill press besides the usual hand tools, using semi-skilled labor.

Results

For use in qualitative analysis, lines may usually be identified by the use of dividers and an accurate millimeter scale. Such measurements are hardly accurate to more than one or two Å but, using good judgment, this is ordinarily close enough to assign a line to the proper element, since only a few elements are present in quantity in plant ash, the rest being represented by only one or two lines or being entirely absent. In case of doubt more accurate measurements can be made with a comparator, but in this case the highest accuracy is not attained because roll film is very light and subject to distortion through changes in humidity. Comparator measurements on thirty-eight lines in a single exposure, made with some precautions to avoid altering the film, had a standard error of $\pm$ 0.4 Å. Since, however, the grating will not resolve lines closer than 0.7 Å in the first order, any attempt to decrease this error would be pointless. When one has become familiar with the appearance of the spectrum given by plant ashes, the characteristic lines of most elements may be recognized readily without recourse to measurements. Figure 2 shows a group of exposures made of ash

[4] Private communication.

samples, with a few lines labeled. The region from C 2478 Å to Cu 3247 Å is shown.

Details of procedures which have been found suitable for quantitative analysis with this instrument will be given in a later paper. Sufficient work has been done to indicate quite conclusively that accuracy of the same order as is obtainable with other instruments may be had by the use of the present one, using the method of measuring line densities and comparing the unknown with an internal standard.

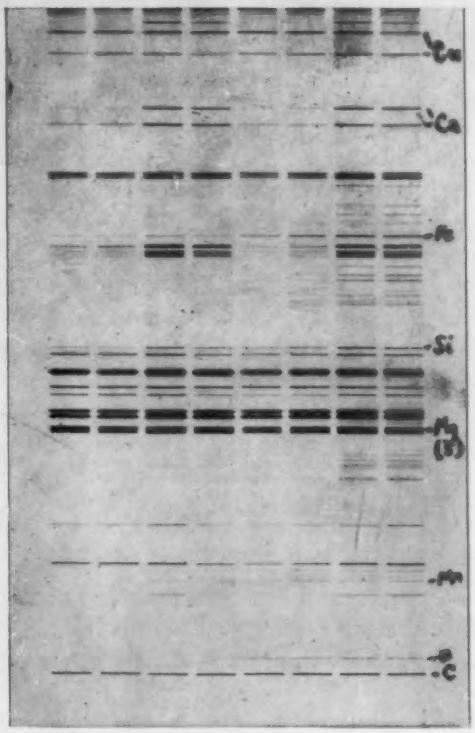

FIG. 2. Sample spectrogram

This spectrograph was designed to make possible rapid and economical analysis. The use of roll film contributes chiefly toward the attainment of these ends. By making possible 24 or 32 exposures before reloading is necessary and by greatly reducing the cost per exposure, it achieves the greatest economy of time and materials. Two hundred fifty to 300 exposures may be made comfortably in an average working day, at a cost of about one cent per exposure for materials.

There are, however, certain limitations in the use of replica gratings which must be taken into account. The intensity and sensitivity obtainable

with these gratings are in no way comparable with that available with a prism spectrograph. In a grating spectrograph the light which falls on the grating is divided among two spectra in each of several orders and a considerable portion of it remains undispersed, going into the direct image of the slit. As a consequence, only about twenty per cent. or less turns up in the spectrum which is recorded.

To this effect must be added the effect of the absorption of light by the material of which the replica is made. The replica has a strong absorption band in the region of 3100 Å which greatly interferes with the use of the 3159 Å calcium line for the determination of that element. This line cannot be used except for samples containing fairly large quantities of calcium. For smaller amounts the calcium lines in the blue must be used. There is also a good deal of absorption in the ultra violet below 2600 Å and lines below 2470 Å are not recorded. The chief effect of this is to reduce the sensitivity of the instrument to the 2497 boron lines, making the determination of small amounts of boron difficult. No difficulty, however, is found with most ash samples since these usually contain enough boron to be easily measurable. Difficulties arise in the analysis of potato tuber ash, which is low in boron and high in potash, which tends to suppress the boron lines; and in the analysis of tracheal sap. Using the high voltage alternating current arc of DUFFENDACK (1), it has been possible to detect 8×10^{-5} milligrams of boron with this instrument. The limiting sensitivity to phosphorus, using the AC arc, is about four times that of boron, 3×10^{-4} milligrams. For all other elements which can be determined spectrographically, the sensitivity is ample for mineral nutrition studies.

Summary

An inexpensive spectrograph has been designed and constructed, using a concave diffraction grating replica to disperse light. This spectrograph has proved satisfactory for the analysis of plant ashes, is rapid, and economical to operate.

Assistance in the preparation of this material was furnished by the personnel of the Work Projects Administration, Official Project no. 65–1–71–140. Sponsor: University of Minnesota. Thanks is due to Mr. FRANK CARTER who did most of the work of constructing the instrument, and who made many helpful suggestions as to rendering the design amenable to construction with the tools available; and to Mr. PHILIP HAMM who suggested the use of roll film.

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BRIEF PAPERS

AN ATMOSPHERIC HALATOMETER*

C. P. Sideris

(WITH ONE FIGURE)

The word halatometer, derived from two Greek words, halas—salt (Nom., ἅλας Gen., ἅλατος) and meter—measure (μέτρον) has been applied to an apparatus intended for the collection of salt particles from the atmosphere.

The measurement of salt water carried away by strong winds from the ocean and deposited on agricultural plants grown in fields situated near the coast becomes at times an important economic problem and deserves serious consideration. In certain of the islands of the Hawaiian archipelago pineapple fields are exposed to trade winds which deposit considerable amounts of salts on plants during the course of a year. Certain plants are more tolerant than others to salt injury. For instance, *Ananas comosus* (L.) Merr. exposed to salt sprays exhibits leaf-tip death which in severe cases involves the entire leaf and retards the rate of growth and period of fructification.

If the amounts of salt deposited on plants by winds are known, studies on prophylactic and corrective measures may be more easily made. For example, the extent and density of plantings of *Casuarina equisetifolia* Linn., a salt tolerant plant, planted on the ocean side of pineapple fields for windbreak and salt trapping purposes, can be estimated better if the amounts of windblown sodium chloride are known. Also the amounts of potassium to be applied for increasing the tolerance of *Ananas comosus* (L.) Merr. to chloride toxicity, in accordance with certain studies (**1**), can be better evaluated after the amounts of windblown sodium chloride have been determined. The instrument, illustrated in figure 1 and described in this paper, was constructed for this purpose. The total amount of salt trapped by the instrument is measured by a chemical analysis of the chloride content of an aliquot. Other methods of analysis, either chemical or physical, can be used for determining the total salt content of the sample.

It consists of a metal box (A) and a bisectional tube (C-1 and C-2). The former is provided with a door and encloses a glass jar (B) which acts as a receiver for salt and water, and two or three graduated cylinders of different capacity. The box acts also as a supporter for the superstructure of the apparatus consisting of a metal rod (D) about ½ inch in diameter and 42 inches in length, and the two sections of the metal tube, an upper (C-1) and a lower one (C-2).

* Published with the approval of the Acting Director as Miscellaneous Paper no. 38 of the Pineapple Research Institute, University of Hawaii.

The metal box (A), with all sides flat and of equal area (4 feet square) excepting the top one which is inclined for rainwater drainage, is made of galvanized iron with a skeleton of angle-iron ($1\frac{1}{2}$ inches wide and $\frac{1}{8}$ inch thick), and lined on the upper side with four pieces of flat iron ($1\frac{1}{2}$ inches wide and $\frac{1}{8}$ inch thick) (11) which are screwed to the angle-iron pieces of the framework at the edges of the box. A fifth flat iron piece (10) ($1\frac{1}{2}$ inches wide and $\frac{1}{8}$ inch thick) in the shape of an inverted arc and supporting

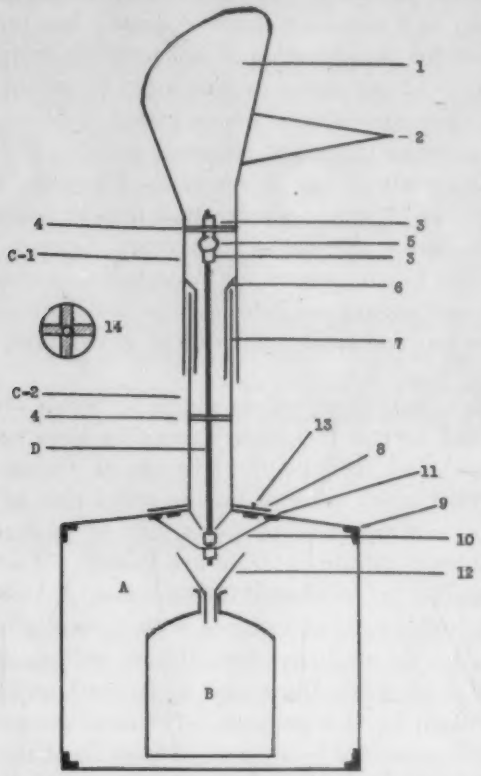

FIG. 1. A, box; B, liquid container; C–1, upper section of tube; C–2, lower section of tube; D, metal rod; (1), funnel of tube; (2), vane; (3), female screw; (4), cross shaped plate; (5), ball bearing; (6), wall of inner tube or sleeve; (7), wall of lower C–2 tube; (8), flanges of C–2 tube; (9), angle iron; (10), rod supporter; (11), flat iron; (12), funnel; (13), bolts; (14), top view of cross shaped plate (4).

the metal rod (D) through a hole 9/16 inch in diameter, is fastened by means of bolts to two of the flat iron pieces of the upper side of the box.

The tube, consisting of the upper (C-1) and lower (C-2) sections, is connected to the upper side of the box by means of lateral flanges (8) soldered to the lower section of the tube and fastened to the upper side of the box by bolts through the flat iron pieces.

The upper section of the tube (C-1), of a greater diameter than the lower one (C-2), is equipped at the upper end with a funnel-like structure which greatly augments its opening area and also its contact area for salt and water from the atmosphere. A vane is attached to the funnel-like structure to direct the opening towards the prevailing winds. The upper section of the tube is also provided with an inner tube (6) which acts as a sleeve for the lower tube (C-2). A brass plate (4) in the shape of a cross and soldered to the inner walls of the tube serves to support the entire weight of the upper section of the tube (C-1). The plate (4), having a hole 9/16 inch in diameter and resting on a ball bearing (5) is held in place by the metal rod (D). A similar plate (4) soldered to the lower section of the tube (C-2) serves to hold the rod (D) in position.

The rod (D) is threaded at the upper end to an extent of 3 and at the lower end of 2 inches. The lower end of the rod is fastened to the flat iron plate of the supporter by means of two female screws, one above and the other below the plate.

Any points on the description of the apparatus not mentioned in the text may be obtained from figure 1.

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EFFECT OF INFECTING CARROT PLANTS WITH CERTAIN VIRUSES ON SEEDSTALK DEVELOPMENT[1]

EL SAYED SAKR AND H. C. THOMPSON

(WITH ONE FIGURE)

In an experiment on the effect of temperature on seedstalk development in carrots there appeared some yellow plants in the greenhouse in 1939–1940 which formed seedstalks earlier than others in the cool ranges of temperatures. In the high temperature ranges (60° to 70°, and 70° to 80° F.) these yellowed plants were the only ones that formed seedstalks under certain treatments. Tests showed that these plants were infected with the virus of aster yellows (Eastern). The question arose: Does this disease bring about a change within the plant that results in seedstalk development? If the disease causes a change in the plants that results in seedstalk development, inoculating the plants with the disease under controlled conditions ought to cause them to go to seed. To obtain information on this point the following experiment was conducted: Seed was sown in flats in a 60°–70° F. greenhouse on December 10, 1940. On January 15, the plants were potted in 6-inch pots and on June 5, 80 plants were selected for uniformity and were in good growing condition. These 80 plants were treated as follows: Ten plants served as a check, that is, they were not inoculated; the remaining 70 plants were divided into lots of 10 plants each. One lot was inoculated with aster yellows using insect carriers, *Macrosteles divisus* (Uhl.) (*Cicadula sexnotata*, Fall.) caging 3 to 4 insects around each plant. The other lots were inoculated with sap extract of aucuba mosaic, latent virus of potato, alfalfa mosaic, cucumber virus 1 (the so-called "white pickle mosaic") and the remaining 20 plants were inoculated with tobacco mosaic in two forms, one as sap extract from a diseased tobacco leaf and the other form was the compounds of the disease, nucleic acid and protein, after they were separated from diseased leaves and added together again without uniting them chemically. These compounds were supplied through the kindness of Dr. V. L. FRAMPTON of the Plant Pathology Department, Cornell University. The inoculation was done by squeezing the leaf between pieces of clean cheesecloth and rubbing the cheesecloth, saturated with sap, on the carrot leaf. For the tobacco mosaic compounds, a piece of cheesecloth was dipped into the mixture of liquid nature after which it was rubbed on the carrot leaf. The inoculated plants were put under a cheesecloth cage to prevent the spread of the diseases.

All the plants treated with aster yellows formed seedstalks but not a single plant given other treatments developed a seedstalk. Figure 1 shows

[1] Paper no. 244, Department of Vegetable Crops. Cornell University, Ithaca, New York.

a carrot plant that formed seedstalks as a result of infection with aster yellows virus. The plant was bushy and had more than one seedstalk.

SMITH (5) and HOLMES (4) did not report the susceptibility of carrots to aucuba mosaic, latent virus of potato, and alfalfa mosaic. The carrot plants, French Forcing, used in this experiment did not show the disease symptoms of these three virus diseases. There are different strains of tobacco mosaic, to some of which carrot may or may not be susceptible (4). Since the plants did not show the disease symptoms, one is justified in saying that the strain used does not infect carrot. SMITH (5) and HOLMES (4), however, list carrot as being susceptible to cucumber mosaic and aster yellows. Cucumber mosaic and aster yellows symptoms showed up. Why one did not cause carrot plants to form seedstalks while the other did is not known. It may be that aster yellows causes a change in the plant, as a result of which a seedstalk develops while cucumber mosaic brings about a different change, thus causing the diseased condition of the plant without forming a seedstalk. This change favoring seedstalk formation may be caused by hormone formation within the plant as postulated by CAILACHJAN (1) and supported later by HAMNER and BONNER (3).

FIG. 1. A carrot plant developing numerous seedstalks as a result of infection with aster yellows virus.

It has been noticed that, although seedstalks may be formed, there was no seed set. Sometimes flowers were formed when the infection took place at a late stage of plant growth, or under cool temperature. If it took

place under high temperature (70°–80° F.) at a very early stage of plant growth, no flowers were formed although the seedstalk developed.

HAMNER (**2**) and THOMPSON (**6**) emphasized the fact that a certain treatment may favor a certain stage in flowering but not necessarily another. One set of environmental conditions for instance may be favorable for flower primordia development, while it may not be so for flower development, or seed set.

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NOTES

Annual Election.—The nineteenth annual election of the American Society of Plant Physiologists has resulted in the election of Dr. WALTER E. LOOMIS, Iowa State College, as president. The vice-president is Dr. DONALD B. ANDERSON, North Carolina State College; elected member of the executive committee, Dr. D. R. HOAGLAND, University of California; and the retiring president, Dr. E. C. MILLER, Kansas State College, has been returned to the editorial board. Difficult days are ahead for all cultural and scientific organizations. It is incumbent upon all of us to place our talents at the disposal of the officers, so that they may be sustained in their efforts to serve the society. There may be difficulties of unforeseen nature in connection with conventions during the war. We hope that our annual conventions may not be interfered with, but it is always possible that travel restrictions may have to be imposed. In any event, members should do anything possible to assist the officers, and to maintain the activities of the society unimpaired.

New England Section.—The New England Section held its annual meeting at Smith College, Northampton, Massachusetts, May 22–23, 1942. There were three sessions for presentation of papers, and one session relating to the rôle that plant physiologists are playing in the war effort. Dr. DOROTHY DAY as Chairman of the Local Committee provided admirable facilities. She was also chairman of the Section. In spite of conflicting commencements and gasoline rationing, twelve institutions were represented. The 1943 meeting will be held at Harvard in Cambridge, Massachusetts. Officers elected are: Chairman, Dr. K. V. THIMANN, of Harvard; vice chairman, Dr. B. E. GILBERT, of Rhode Island State; and secretary-treasurer, Dr. L. H. JONES, of Massachusetts State.

Purdue Section.—It is a pleasure to report the activities of the Purdue Section of A.S.P.P. for the 1941–42 season. Sixteen meetings were held between October 14, 1941, and May 10, 1942. The first and last meetings of the year are usually dinner meetings, with outside speakers. On October 14 Dr. E. R. MARTELL of the Purdue Forestry Department discussed Reforestation in the United States. At the last meeting, on May 10, 1942, Dr. YUNCKER, head of the Botany Department of DePauw University gave an illustrated lecture, A Trip Through Samoa. The meeting of February 11 featured a round table discussion of the meetings at Dallas. The speakers for the regular meetings were: Dr. A. J. ULLSTRUP, Dr. G. L. JENKINS, Dr. G. D. SCARSETH, Dr. G. E. MARSHALL, Dr. R. M. CALDWELL, Dr. ALICE P. WITHROW, Dr. W. S. GILLAM, Dr. J. L. ROBERTS, Dr. F. P. ZSCHEILE, and Mr. E. R.

CLARK, Mr. N. K. ELLIS, and Mr. B. A. KRANTZ. The topics presented were all of considerable interest, and the section year was a very satisfactory one. The chairman during the last year was Dr. F. P. ZSCHEILE, and the secretary-treasurer Dr. WENDELL R. MULLISON. For the coming year Dr. ALICE WITHROW will be chairman, and Professor C. O. LEE, secretary-treasurer.

Southern Section.—This brief note about the Southern Section is to call attention to a very valuable service which this section has been rendering to its members. After the annual meeting, the Section has had the proceedings of the round table discussion on *Further Aspects of Plant Nutrition* mimeographed and sent to all members. It is a very fine service, one which might be emulated by other sections. The transcript from the 1942 round table discussion occupies 31 pages, and gives the salient features of the discussions in a form suitable for permanent preservation. This is a very commendable contribution by the Southern Section.

Benjamin Minge Duggar.—At the Dallas Meeting the American Society of Plant Physiologists authorized the dedication of the July, 1942, number of PLANT PHYSIOLOGY to Dr. BENJAMIN M. DUGGAR, of the University of Wisconsin, in celebration of the seventieth anniversary of his birth, which will occur on September 1, 1942. Dr. DUGGAR's career is so well known among his colleagues, so much admired, and so deeply appreciated that it is unnecessary to record it at this time. Born at Gallion, Alabama, September 1, 1872, he received his early education in southern institutions, then transferred to Harvard, and Cornell, where he received his Ph.D. in 1898. He was among the youngest members of that distinguished group of American botanists, the one hundred starred men of the first edition of American Men of Science. Of that original group there are still 39 survivors, only four of which are still under 70 years of age.

It is not only a pleasure, but a very great privilege, to carry out the instructions of the Society as expressed at Dallas, to dedicate this number in his honor, and to extend to him and his family, on behalf of all members of the Society, our felicitations, and our good wishes on the occasion of the seventieth anniversary to be celebrated on September 1. We hope that he may long remain in the group of elder botanical statesmen, whose ages run from 70 to 89 at the present time, with health and full vigor to the end of his years.

It is also appropriate to express our sincere thanks for Dr. DUGGAR's cooperation in obtaining the portrait with signature which forms the frontispiece to this number.

Annual Review of Biochemistry.—After a decade of distinguished service to the field of biochemistry, the *Annual Review of Biochemistry* begins

the second decade with volume XI. Throughout this period it has been the most valuable single contribution to progress in this field. It has become *the* indispensable tool for investigators of metabolism.

Volume XI contains 26 progress reports, most of which are of interest to both plant and animal physiologists; for even though the summaries concern in some cases only animal studies, yet they are valuable to students of plant life who must be comparative physiologists in much of their work. It is not possible to review the individual papers, but special attention should be directed to some of them. Of great interest is the review of M. L. HUGGINS, X-ray Studies of the Structure of Compounds of Biochemical Interest. This paper will repay a careful reading and study. C. W. SHOPPEE has reviewed the Chemistry of the Steroids. This reveals the amazing advances that have been made in this important field. S. HECHT's Chemistry of Visual Substances will be of general interest. Among the reviews of special interest to the plant physiologist may be mentioned A. H. K. PETRIE's Mineral Nutrition of Plants; P. R. WHITE on Plant Tissue Cultures; R. J. DUBOS on Microbiology, H. HIBBERT on Lignin, and L. C. CRAIG on the Alkaloids. E. G. BALL's review of Biological Oxidations and Reductions is of general physiological interest. Other reviews cover the fields of hormone and vitamin chemistry, non-proteolytic hydrolytic enzymes, acyclic constituents of fats, proteins and amino acids, metabolism of phosphorus compounds, metabolism of fats, carbohydrates, proteins and amino acids, etc. A few of the reviews are strictly in the animal field, such as the Chemistry of Muscle, Avian Biochemistry, Animal Pigments, Immunochemistry, etc.

The reviews are generally very readable, give the true trends of current research, and stimulate renewed attack upon disputed problems. The reviewer considers this series of volumes a contribution of the first importance, one which deserves undivided support of biochemists. Nothing but mass production could make possible a volume of this size (736 pages) at the low price of $5.00 per copy. Orders should be addressed to Annual Reviews Inc., Stanford University, California.

Annual Review of Physiology.—The fourth volume of this admirable series contains 23 reviews. It deals largely with animal physiology, but such papers as L. R. BLINKS on Permeability; J. P. PETERS on Water Metabolism; G. S. AVERY on Growth; H. H. STRANDSKOV on Physiological Aspects of Genetics; C. P. RICHTER on Physiological Psychology; and M. I. SMITH on the Physiology of Drug Addiction appeal to a much wider audience than the animal physiologists. In its field the *Annual Review of Physiology* is performing a very valuable service, and helping to maintain balanced perspective among research men. It is naturally very difficult to select the material for review, for there are hundreds of papers which cannot receive

attention in the review of any one year. Nevertheless, the careful and judicious handling of the topics, the conscientious attitude of the reviewers and editors, keeps the work at a high level of excellence. This volume is also obtainable of Annual Reviews Inc., Stanford University, California at $5.00 per copy. It contains 709 pages, and is well indexed.

Vitamins.—An impressive volume on the Chemistry and Physiology of the Vitamins by Dr. H. R. ROSENBERG, of the Jackson Laboratory, E. I. du Pont de Nemours & Co., has been published by Interscience Publishers, Inc., New York City. The author has worked in the laboratories of RUZICKA and REICHSTEIN in Switzerland, and has had long experience in the field of vitamin chemistry. The work was conceived about 10 years ago, and aims to give a comprehensive treatment of the chemistry and physiology of all the known vitamins.

The first section of the work is general, concerned with definitions, history, nomenclature, occurrence, isolation, constitution, synthesis, preparation, biogenesis, specificity, determination, standards, etc. The metabolism of vitamins in plants and animals, physiological action of the vitamins, the general mechanism of action, and relation of vitamins to each other, and to hormones and minerals are briefly treated in this introductory chapter. The pathological aspects, avitaminosis, hypovitaminosis, and hypervitaminosis, are introduced, along with a discussion of the general requirements for health.

The following sections treat groups of vitamins or single ones, in detail. The various A vitamins, vitamin B_1, B_2, B_6, nicotinic acid (and nicotinamide), pantothenic acid, inositol, para-amino-benzoic acid, ascorbic acid (C), the group of D vitamins, the E vitamins, biotin (H), the vitamins K, vitamin P, and non-identified vitamins, all receive full treatment. The appendix considers essential fatty acids, amino-acids, carbohydrates, and choline and its relatives, as "vitagens". The last 60 pages of the text deals with the patents, a patent index, of the methods of preparation, etc. The author index of 44 pages, three columns to the page, gives evidence of the enormous amount of work done in the field. There is also a useful subject index.

The book is very substantial, 674 pages, 25 text figures, and copiously supplied with chemical formulae, giving structural features, and chemistry of preparation. It should be a very useful reference work to the biochemists and plant and animal physiologists. The price is $12.00 per copy. Those who cannot own it should have libraries obtain it so that the information may be available to research workers.

Minor Elements.—The third supplement to the third edition of the *Bibliography of References to the Literature on the Minor Elements and their Relation to Plant and Animal Nutrition* has been published by the Chilean

Nitrate Educational Bureau, Inc., a service for which all plant physiologists are most grateful. As usual, the abstracts are arranged in alphabetical order according to the elements, from aluminum to zinc. The three indexes are exceedingly useful. Under each element is given the names of the authors who have worked upon it; forty-seven of the elements are included in this index. The botanical index lists the contributions which deal with each species mentioned. More than 100 species are listed (common names). About 750 authors have contributed the annual advance in the field.

The Chilean Nitrate Educational Bureau has pursued the enlightened policy of distributing these bibliographies upon request, and every member of our organization should maintain this series of publications on the personal library shelf. It is one of the most useful annuals we have, and we trust that the policy of providing annual supplements may be continued indefinitely. For those who do not know the address, we are glad to mention that Mr. HERBERT C. BREWER is the Director of the Bureau, and that the address is 120 Broadway, New York.

Plant Biology.—An elementary text book of botany, by Dr. PAUL WEATHERWAX, Professor of Botany at Indiana University. While it is intended for first year college students, it is really elementary; perhaps one should not say too elementary, for it is difficult to find anything too elementary for freshmen these days. It is entertaining, well written, and is the result of long experience of Dr. WEATHERWAX as teacher and friend of students.

The presentation occupies 27 chapters, begins with common considerations of the cell, and of the plant as a functioning whole. Beginning at chapter 18, the customary survey of the phyla begins, algae, fungi, bryophyta, pterodophyta, etc., and the closing note is on the problems of out-door biology: evolution, pollination, dormancy and dispersal, migration, communities, succession.

The book closes with the glossary so frequently included in beginning textbooks to help with the conglomeration of terms with which botany is blessed, and an index. The reviewer would expect that many teachers would want to supplement the text with more details where explanations stop far short of what we know about the life processes of plants. He has demanded more of beginning students than this work demands, and believes that students can, and will, do more if it is demanded of them. We face the problem of getting farther with each course, or of taking too long for education, or of having to consider the same processes repeatedly, delving deeper each time, until the student has adequate equipment to understand the present state of our knowledge. The treatment of respiration is a case in point. How many excursions must we make into it to reach the current research stage,

and how far should we go for people to be adequately educated about the process? Our feeling is that the student might profitably go a good deal farther the first time he comes to it in college, than this text takes him.

The work has 455 pages, and may be obtained from W. B. Saunders Co., Philadelphia, at $3.25 per copy.

Ecological Crop Geography.—This work by K. H. W. KLAGES, agronomist at the University of Idaho, is of considerable interest because few writers have gone into this phase of ecology, or geography. The first division of the book considers various social factors, historical, population problems, factors determining world centers of population, social environments, etc. The second division deals with the physiological factors of environment; the third with ecological factors; and the final one with the geographic distribution of various crop plants. It is full of valuable information, and should stimulate wider interest in these geographic aspects of crops and vegetation in general.

It contains 32 chapters, 615 pages, and is well illustrated. The publishers, The Macmillan Co., New York, offer it at $4.50 per copy.